L22: Entry 10 of 18 File: USPT Aug 3, 1999

DOCUMENT-IDENTIFIER: US 5932422 A
TITLE: Modulation of drug resistance via ubiquitin carboxy-terminal hydrolase

DATE FILED (1):
19971114

Detailed Description Paragraph Right (10):
To obtain a probe suitable for assessing UCH mRNA expression, those of ordinary skill in the art can obtain the known sequence of a UCH-encoding nucleic acid molecule from publications (e.g., see Day et al., FEBS Lett. 210:157-160, 1987) or by searching the GenBank database accessible through the Internet address: http://www.ncbi.nlm.nih.gov/). To move directly to the sequence of a human mRNA for UCH (formerly referred to as protein gene product (PGP) 9.5), those of ordinary skill in the art can search GenBank using the Accession Number X04741.

Other Reference Publication (7):
Day et al., FEBS Lett., 210:157-160 (1987) Molecular cloning of cDNA coding for human PGP 9.5 protein.

Other Reference Publication (22):
Wilkinson et al., Science 246:670 (1989) Neuron-specific Protein PGP 9.5 is a Ubiquitin Carboxyl-Terminal Hydrolase.

CLAIMS:

4. A method of identifying a compound that modulates drug resistance, the method comprising

(a) determining the level of activity of ubiquitin carboxy-terminal hydrolase in a cell in the presence of a test compound,

(b) comparing the level of activity of ubiquitin carboxy-terminal hydrolase in said cell in the presence and absence of said compound,

(c) identifying said compound as a modulator of drug resistance when the level of ubiquitin carboxy-terminal hydrolase activity is decreased in the presence of said compound.

11. The method of claim 4, further comprising measuring the drug resistance of said cell in the presence of said modulator identified in step (c).

13. A method of identifying a compound that modulates drug resistance, the method comprising

(a) determining the level of expression of ubiquitin carboxy-terminal hydrolase in a cell in the presence of a selected compound,

(b) comparing the level of expression of ubiquitin carboxy-terminal hydrolase in said cell in the presence and absence of said selected compound.

(c) identifying said compound as a modulator of drug resistance when the level of ubiquitin carboxy-terminal hydrolase activity is decreased in the presence of said compound.

20. The method of claim 13, further comprising measuring the drug resistance of said

cell in the presence of said modulator identified in step (c).

22. The method of claim 13 wherein measuring the level of expression of ubiquitin carboxy-terminal hydrolase comprises measuring the level of mRNA encolding the ubiquitin carboxy-terminal hydrolase.

24. A method of identifying a compound that modulates drug resistance, the method comprising

(a) determining whether a selected compound specifically binds ubiquitin carboxy-terminal hydrolase, and

(b) identifying said selected compound as a modulator of drug resistance if said compound selectively binds ubiquitin carboxy-terminal hydrolase.

27. The method of claim 24, further comprising measuring the drug resistance of a cell in the presence of said modulator identified in step (b).

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L22: Entry 4 of 18 File: USPT Jan 9, 2001

DOCUMENT-IDENTIFIER: US 6171786 B1
TITLE: Methods for preventing multidrug resistance in cancer cells

DATE FILED (1):
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Abstract Paragraph Left (1):
This invention is directed to methods for preventing the emergence of multidrug resistance in tumor cells during cancer chemotherapy. In particular, it relates to the use of cytoplasmic calcium antagonists and calmodulin inhibitors, phosphoinositol-dependent phospholipase C inhibitors, and substances that inhibit activation of the transcription factor NF-.kappa.B to prevent the induction of expression of the multidrug resistance gene (MDR1) encoding P-glycoprotein by chemotherapeutic drugs. MDR1 expression, which results in tumor cell resistance to subsequent treatment with certain chemotherapeutic drugs, is shown herein to be induced in response to treatment with various cytotoxic agents, including such agents that are or are not substrates for P-glycoprotein-mediated efflux from cancer cells. Cytoplasmic calcium antagonists and calmodulin inhibitors, phosphoinositol-dependent phospholipase C inhibitors, and substances that inhibit activation of the transcription factor NF-.kappa.B are shown herein to suppress this cellular response. The invention also provides methods for identifying cytoplasmic calcium antagonists and calmodulin inhibitors, phosphoinositol-dependent phospholipase C inhibitors, and substances that inhibit activation of the transcription factor NF-.kappa.B that suppress induction of MDR1 gene expression by cytotoxic drugs. Thus, the invention provides useful methods and reagents for preventing the emergence of multidrug resistance in tumor cells treated with cytotoxic and chemotherapeutic drugs in cancer patients undergoing chemotherapy, when cytoplasmic calcium antagonists and calmodulin inhibitors, phosphoinositol-dependent phospholipase C inhibitors, and substances that inhibit activation of the transcription factor NF-.kappa.B are administered prior to or simultaneously with cytotoxic drug treatment in such individuals.

Brief Summary Paragraph Right (2):
This invention is directed to methods for preventing the emergence of multidrug resistance in tumor cells during cancer chemotherapy. In particular, it relates to the use of inhibitors of particular pathways of signal transduction to prevent the induction of the multidrug resistance (MDR1) gene by chemotherapeutic drugs. MDR1 gene expression, which results in tumor cell resistance to subsequent treatment with certain chemotherapeutic drugs is shown herein to be induced in response to treatment with various cytotoxic agents. Inhibitors of protein kinases, cytoplasmic calcium antagonists and calmodulin inhibitors, phosphoinositol-dependent phospholipase C inhibitors, and substances that inhibit activation of the transcription factor NF-.kappa.B (each of which have been implicated in intracellular eukaryotic signal transduction) are also shown herein to suppress this cellular response. Therefore, such inhibitors are useful in preventing MDR1 induction by chemotherapeutic drugs in a variety of tumor cells, when administered prior to and/or simultaneously with cytotoxic drug treatment in cancer patients.

Brief Summary Paragraph Right (4):
Chemotherapy is a primary form of conventional cancer treatment. However, a major problem associated with cancer chemotherapy is the ability of tumor cells to develop resistance to the cytotoxic effects of anti-cancer drugs during the course of treatment. It has been observed that tumor cells can become simultaneously resistant to several chemotherapeutic drugs with unrelated chemical structures and mechanisms of action. This phenomenon is referred to as multidrug resistance. The best documented and clinically relevant mechanism for multidrug resistance in tumor cells is correlated

with the expression of P-glycoprotein, the product of the MDR1 gene.

Brief Summary Paragraph Right (6):
Human P-glycoprotein is expressed in several types of normal epithelial and endothelial tissues (Cordon-Cardo et al., 1990, J. Histochem. Cytochem. 38: 1277-1287; Thiebaut et al., 1989, Proc. Natl. Acad. Sci. USA 84: 7735-7738), as well as in hematopoietic stem cells (Chaudhary and Roninson, 1991, Cell 66: 85-94), and a subpopulation of mature lymphocytes (Neyfakh et al., 1989, Exp. Cell Res. 185: 496-505). More importantly, MDR1 mRNA or P-glycoprotein have been detected in most types of human tumors, both before and after chemotherapeutic treatment (Goldstein et al., 1989, J. Natl. Cancer Inst. 81: 116-124; Noonan et al., 1990, Proc. Natl. Acad. Sci. USA 87: 7160-7164). The highest levels of MDR1 expression are usually found in tumors derived from MDR1-expressing normal tissues; e.g., renal, adrenocortical or colorectal carcinomas. In other types of solid tumors and leukemias, MDR1 expression prior to treatment is usually relatively low or undetectable, but a substantial fraction of such malignancies express high levels of MDR1 after exposure to chemotherapy (Goldstein et al., 1989, ibid.). Prior to the present invention, the increase in MDR1 expression after chemotherapy was believed to result from in vivo selection for rare, pre-existing tumor cells that were already inherently resistant to chemotherapeutic drugs due to MDR1 expression.

Brief Summary Paragraph Right (7):
Even low levels of MDR1 expression have been correlated with the lack of response to chemotherapy in several different types of cancer (Chan et al., 1990, J. Clin. Oncol. 8: 689-704; Chan et al., 1991, N. Engl. J. Med. 325: 1608-1614; Musto et al., 1991, Brit. J. Haematol. 77: 50-53), indicating that P-glycoprotein-mediated multidrug resistance represents an important component of clinical drug resistance. Whereas many clinical and pre-clinical studies have addressed pharmacological strategies for inhibiting P-glycoprotein function (Ford and Hait, 1990, Pharmacol. Rev. 42: 155-199), prior to the present invention, little was known about the factors that are responsible for the induction or up-regulation of P-glycoprotein expression in tumor cells under conditions relevant to cancer chemotherapy. Understanding such factors provides insight into the development of methods for preventing the appearance of P-glycoprotein in human tumors, thus reducing the incidence of multidrug resistance in cancer, and leading to more effective chemotherapy of cancer.

Brief Summary Paragraph Right (8):
Numerous gene transfer studies have demonstrated that elevated expression of the MDR1 gene is sufficient to confer the multidrug resistance phenotype (Roninson, 1991, ibid.). For instance, mouse NIH 3T3 cells infected with a recombinant retrovirus carrying human MDR1 cDNA became multidrug-resistant in proportion to the density of human P-glycoprotein on their surface; the correlation was not affected by the presence or absence of cytotoxic selection (Choi et al., 1991, Proc. Natl. Acad. Sci. USA 88: 7386-7390).

Brief Summary Paragraph Right (10):
Several laboratories have investigated the factors that regulate MDR1 gene expression in normal and malignant cells. One example of normal physiological regulation of an MDR1 homolog was found in mouse uterine endometrium, where the expression of a mouse mdr gene was induced by steroid hormones at the onset of pregnancy (Arceci et al., 1990, Molec. Repro. Dev. 25: 101-109; Bates et al., 1989, Molec. Cell. Biol. 9: 4337-4344). In rat liver, the expression of an mdr gene was found to be inducible by several carcinogenic or cytotoxic xenobiotics; similar induction was also observed during liver regeneration (Fairchild et al., 1987, Proc. Natl. Acad. Sci. USA 84: 7701-7705; Thorgeirsson et al., 1987, Science 236: 1120-1122). Further, a rodent homolog of MDR1 was induced in several cell lines in response to treatment with certain cytotoxic drugs (Chin et al., 1990, Cell Growth Diff. 1: 361-365). In contrast, no induction of the human MDR1 gene by cytotoxic drugs was detected in any of the human cell lines tested in the same study. Other investigators have also failed to detect MDR1 induction upon treatment with cytotoxic drugs (Schinkel and Borst, 1991, Sem. Cancer Biol. 2: 213-226).

Brief Summary Paragraph Right (11):
Several studies have indicated, however, that the human MDR1 gene may be susceptible to stress induction, under certain conditions. Thus MDR1 expression in some human cell lines was increased by treatment with heat shock, arsenite (Chin et al., 1990, J. Biol. Chem. 265: 221-226) or certain differentiating agents (Mickley et al., 1989, J. Biol. Chem. 264: 18031-18040; Bates et al., ibid.). Some cytotoxic P-glycoprotein substrates were reported to stimulate transcription of a reporter gene from the human MDR1 promoter (Kohno et al., 1989, Biochem. Biophys. Res. Commun. 165: 1415-1421; Tanimura

et al., 1992, Biochem. Biophys. Res. Commun. 183: 917-924) and to increase P-glycoprotein expression in a mesothelioma cell line after prolonged exposure (Light et al., 1991, Int. J. Cancer 49: 630-637). Despite such reports of MDR1 induction, however, it has never been determined whether short-term exposure to any agents used in cancer chemotherapy could induce expression of the MDR1 gene in human cells, and whether MDR1 induction could be prevented.

Brief Summary Paragraph Right (12):
Recently, Kiowa et al. (1992, FEBS Lett. 301: 307-309) have reported that the addition of a flavonoid, quercetin, can prevent an increase in MDR1 expression in a hepatocarcinoma cell line induced by arsenite, a compound which is not used in cancer treatment, but is known to activate the transcriptional pathway mediated by the heat shock response element in the MDR1 promoter. Although not disclosed by Kiowa et al., inhibition of PKC activity is one of the biological effects of quercetin (Gschwendt et al., 1984, Biochem. Biophys. Res. Commun. 124: 63). It is possible therefore that PKC inhibition by quercetin could be responsible, in part, for the observed inhibition of MDR1 induction by arsenite. However, it is noteworthy that the ability of quercetin to inhibit a transcriptional response mediated by the heat shock response element is believed to those skilled in the art to be unrelated to PKC inhibition. (see, e.g., Kantengwa and Polla, 1991, Biochem. Biophys. Res. Commun. 180: 308-314). Furthermore, Kiowa et al. provide no suggestion that non-flavonoid PKC inhibitors would be able to inhibit MDR1 induction by arsenite, or that quercetin would be able to inhibit the induction of MDR1 expression when used in combination with chemotherapeutic drugs or any other agents that are not known to activate the heat shock response element-mediated pathway.

Brief Summary Paragraph Right (13):
In addition to MDR1, another pleiotropic drug transporter has been recently discovered (Grant et al., 1994, Cancer Res. 54: 357-361)). This protein, termed the Multidrug Resistance-associated Protein (MRP), has been shown to confer a pattern of resistance to cytotoxic, particularly chemotherapeutic, drugs similar to the P-glycoprotein transporter encoded by the MDR1 gene. No inhibitors of MRP expression have been previously reported.

Brief Summary Paragraph Right (15):
This invention is based, in part, on the discovery that anticancer drugs, whether or not transported by P-glycoprotein, can induce the expression of the MDR1 gene in human tumor cells of diverse tissue origins. The increase in MDR1 gene expression is observed at both the mRNA and protein levels. MDR1 induction is also observed upon treatment of cells with PKC agonists. Further, this induction by either a cytotoxic drug or a PKC agonist can be prevented by treatment of cells with a protein kinase inhibitor, indicating that a protein kinase-mediated pathway is involved in MDR1 gene induction, and that protein kinase inhibitors may be useful in preventing the expression of MDR1 gene in cancer cells exposed to chemotherapeutic agents. More specifically, this inhibitory effect is associated with inhibition of PKC, since protein kinase inhibitors that are inactive against PKC fail to suppress MDR1 induction, while protein kinase inhibitors which have potent effects on PKC efficiently inhibit the response.

Brief Summary Paragraph Right (16):
In addition, certain other substances which interfere with signal transduction in eukaryotic, particularly mammalian, cells have been found to prevent MDR1 induction by cytotoxic drugs or PKC agonists. These include cytoplasmic calcium antagonists and calmodulin inhibitors, phosphoinositol-dependent phospholipase C inhibitors, as well as substances that inhibit activation of the transcription factor NF-.kappa.B.

Brief Summary Paragraph Right (17):
The ability of chemotherapeutic drugs to induce MDR1 expression in human cells upon short-term exposure in vitro indicates that cancer chemotherapy induces multidrug resistance directly, rather than through selection of pre-existing rare variants. Such direct induction is likely to occur during a patient's course of drug treatment, and it would account, at least in part, for the observed increased incidence of MDR1 expression in treated relative to untreated malignancies. Hence, administration of signal transduction inhibitors as disclosed herein prior to and/or simultaneously with the chemotherapy involving cytotoxic drugs may be useful in preventing MDR1 induction, and thus prevent the emergence of multidrug resistant cancer cells, leading to a more favorable therapeutic outcome.

Brief Summary Paragraph Right (18):
The invention is illustrated by way of examples in which PKC agonists are shown to

induce MDR1 expression in normal peripheral blood lymphocytes (PBL) and tumor cells. Additionally, various cytotoxic anticancer drugs are also described to be capable of activating the MDR1 gene. Importantly, protein kinase inhibitors, cytoplasmic calcium antagonists and calmodulin inhibitors, phosphoinositol-dependent phospholipase C inhibitors, and inhibitors of transcription factor NF-.kappa.B activation, are shown herein to prevent this MDR1 induction mediated by PKC agonists or cytotoxic drugs, especially in tumor cells that have little or no detectable P-glycoprotein prior to treatment.

Brief Summary Paragraph Right (20):
In another aspect, the present invention provides a method for decreasing multidrug resistance in cancer cells, and an in vitro method for identifying protein kinase inhibitors which would be useful towards this goal. This aspect of the invention is based, in part, on the discovery that certain protein kinase inhibitors can inhibit expression of a multidrug-resistance associated protein, termed MRP (Grant et al., 1994, ibid.), in cancer cells that express this protein in the absence of the inhibitor. This aspect of the invention is shown by way of examples in which protein kinase inhibitors are shown to inhibit MRP expression in tumor cells expressing this protein. A variety of uses are encompassed by this aspect of the invention as described herein, including but not limited to, decreasing expression levels of this protein in multidrug resistant tumor cells during cancer chemotherapy.

Drawing Description Paragraph Right (7):
FIG. 2. cDNA-PCR analysis of the effects of TPA, DOG and staurosporine on MDR1 mRNA expression in different cell lines. In each lane, the upper band (167 bp) corresponds to MDR1, and the lower band (120 bp) to .beta..sub.2 -microglobulin specific PCR products.

Drawing Description Paragraph Right (8):
FIG. 2A: Effects of TPA or DOG, with or without staurosporine treatment, on MDR1 mRNA expression in H9 cells.

Drawing Description Paragraph Right (9):
FIG. 2B: Time course of induction of MDR1 mRNA in H9 cells by TPA. The two negative control (neg. con.) lanes correspond to PCR carried out with water or reverse transcriptase mixture without RNA in place of cDNA.

Drawing Description Paragraph Right (10):
FIG. 2C: Induction of MDR1 mRNA in K562 cells by TPA or DOG and in MCF-7 cells by TPA.

Drawing Description Paragraph Right (11):
FIG. 3. Flow cytometric analysis of drug-induced MDR1 expression.

Drawing Description Paragraph Right (15):
FIG. 4. cDNA-PCR analysis of MDR1 mRNA expression in drug-treated cells. In each lane, the upper band (167 bp) corresponds to MDR1, and the lower band (120 bp) to .beta..sub.2 -microglobulin specific PCR products, amplified in separate tubes.

Drawing Description Paragraph Right (16):
FIG. 4A. MDR1 induction in K562 cells by Ara-C. Cells were exposed to the indicated concentrations of Ara-C for 4.5 days. Cell growth relative to untreated cells was determined by the MTT assay in parallel with RNA extraction.

Drawing Description Paragraph Right (17):
FIG. 4B. MDR1 induction in K562 cells treated with different drugs. The times of drug exposure are indicated. The drugs and their concentrations are as follows: (-), untreated cells; DAU, 250 ng/mL daunorubicin; ADR, 500 ng/mL Adriamycin; VBL, 20 ng/mL vinblastine; VP, 1 .mu.g/mL etoposide; MTX, 200 ng/mL methotrexate; CDDP, 3 .mu.g/mL cisplatin; CHL, 50 .mu.M chlorambucil; 5FU, 2 .mu.g/mL 5-fluorouracil; HU, 30 .mu.M hydroxyurea.

Drawing Description Paragraph Right (18):
FIG. 4C. MDR1 induction in KB-3-1 carcinoma cells, untreated or treated for 2 days with 200 ng/mL Adriamycin or 10 .mu.M Ara-C.

Drawing Description Paragraph Right (19):
FIG. 4D. MDR1 induction in EJ carcinoma cells, untreated (-) or treated for 4 days with 10 .mu.M Ara-C.

Drawing Description Paragraph Right (20):
FIG. 4E. Maintenance of drug induced MDR1 expression in K562 cells. Cells were treated for 3 days with 60 ng/mL Adriamycin, 10 .mu.M Ara-C or 200 ng/mL methotrexate and cultured in drug-free medium for the indicated period of time.

Drawing Description Paragraph Right (21):
FIG. 5. Effect of protein kinase inhibitors on MDR1 mRNA induction by cytotoxic drugs in H9 cells. In each experiment, the inhibitors staurosporine (ST), H7, Iso-H7 (IH7) or HA1004 (HA) were added twice, the first time immediately prior to the addition of the corresponding drug and the second time after the specified period of time.

Drawing Description Paragraph Right (28):
FIG. 7. cDNA-PCR analysis of inhibition by protein kinase inhibitors of MDR1 mRNA expression induced by treatment with Ara-C or TPA. In each lane, the upper band (167 bp) corresponds to MDR1, and the lower band (120 bp) to .beta..sub.2 -microglobulin specific PCR products, amplified in separate tubes.

Drawing Description Paragraph Right (29):
FIG. 7A. Effect of tyrphostins on MDR1 induction in H-9 cells by Ara-C. Cells were incubated for 10 hours with 25 .mu.M Ara-C (Lanes 3 and 4) or with Ara-C and 50 .mu.M tyrphostin A25 (Lanes 5 and 6) or with Ara-C and 50 .mu.M tyrphostin B46 (Lanes 7 and 8). Each of the tyrphostins were added to their respective H-9 cell cultures 16 hours prior to the addition of Ara-C. Negative control lanes (Lanes 1 and 2) represent H-9 cell cultures incubated overnight in the absence of Ara-C and either protein kinase inhibitor.

Drawing Description Paragraph Right (30):
FIG. 7B. Effect of neomycin sulfate on MDR1 induction in H-9 cells by Ara-C. Cells were incubated for 10 hours with 25 .mu.M Ara-C (Lanes 3 and 4) or with Ara-C and 4 mM (Lanes 5 and 6) or 10 mM (Lanes 7 and 8) neomycin sulfate. Neomycin sulfate at each concentration was added to the respective H-9 cell cultures 45 minutes prior to the addition of Ara-C. Negative control lanes (Lanes 1 and 2) represent H-9 cell cultures incubated overnight in the absence of Ara-C and neomycin sulfate.

Drawing Description Paragraph Right (31):
FIG. 7C. Effect of an erbstatin analog on MDR1 induction in H-9 cells by Ara-C. Cells were incubated for 10 hours with 25 .mu.M Ara-C (Lanes 3 and 4) or with Ara-C and the erbstatin analog methyl-2,5-dihydroxycinnamate at a concentration of 32 .mu.M (Lanes 5 and 6) or 64 .mu.M (Lanes 7 and 8). The erbstatin analog at each concentration was added to the respective H-9 cell cultures 45 minutes prior to the addition of Ara-C. Negative control lanes (Lanes 1 and 2) represent H-9 cell cultures incubated overnight in the absence of Ara-C and either protein kinase inhibitor.

Drawing Description Paragraph Right (32):
FIG. 7D. Effect of calphostin C on MDR1 induction in H-9 cells by Ara-C. Cells were incubated for 10 hours with 25 .mu.M Ara-C (Lanes 3 and 4) or with Ara-C and calphostin C at a concentration of 0.1 .mu.M (Lanes 5 and 6) or 1 .mu.M (Lanes 7 and 8). Calphostin C at each concentration was added to the respective H-9 cell cultures 45 minutes prior to the addition of Ara-C, and plates were incubated under direct illumination by white light. Negative control lanes (Lanes 1 and 2) represent H-9 cell cultures incubated overnight in the absence of Ara-C and either protein kinase inhibitor.

Drawing Description Paragraph Right (33):
FIG. 7E. Effect of chelerythrine on MDR1 induction in H-9 cells by Ara-C. Cells were incubated for 10 hours with 25 .mu.M Ara-C (Lanes 3 and 4) or with Ara-C and chelerythrine at a concentration of 1 .mu.M (Lanes 5 and 6) or 5 .mu.M (Lanes 7 and 8). Chelerythrine at each concentration was added to the respective H-9 cell cultures 45 minutes prior to the addition of Ara-C. Negative control lanes (Lanes 1 and 2) represent H-9 cell cultures incubated overnight in the absence of Ara-C and either protein kinase inhibitor.

Drawing Description Paragraph Right (34):
FIG. 7F. Effect of staurosporine and an erbstatin analog on MDR1 induction in H-9 cells by TPA. Cells were incubated overnight with TPA (10 ng/mL, Lanes 3 and 4) or with TPA and the erbstatin analog methyl-2,5-dihydroxycinnamate (32 .mu.M, Lanes 5 and 6) or TPA and staurosporine (30 nM, Lanes 7 and 8). The erbstatin analog or staurosporine were each added to their respective H-9 cell cultures 45 minutes prior to the addition of TPA. Negative control lanes (Lanes 1 and 2) represent H-9 cell cultures incubated

overnight in the absence of TPA and either protein kinase inhibitor.

Drawing Description Paragraph Right (35):
FIG. 8. cDNA-PCR analysis of inhibition by cytoplasmic calcium antagonists and calmodulin inhibitors, phosphoinositol-dependent phospholipase C inhibitors, and inhibitors of transcription factor NF-.kappa.B activation of MDR1 mRNA expression induced by treatment with Ara-C or any of a variety of cytotoxic drugs. In each lane, the upper band (167 bp) corresponds to MDR1, and the lower band (120 bp) to .beta..sub.2 -microglobulin specific PCR products, amplified in separate tubes.

Drawing Description Paragraph Right (36):
FIG. 8A. Effect of phosphoinositol-dependent phospholipase C inhibitor 1-(6-((1.beta.-3-methoxyestra-1,3,5(10)-trien- 17-yl)amino)hexyl)-1-H-pyrrole-2,5-dione (U73122) on AraC-induced MDR1 activation. H9 cells were treated (lanes 2 and 3) or untreated (lane 1) with 25 .mu.M Ara C in the absence of (lane 2) or in the presence of 1 .mu.M (lane 3) or 10 .mu.M (lane 4) of U 73122 for 10 hrs at 37.degree. C., 5% CO.sub.2 in a humidified atmosphere. The inhibitor was added 45 min. prior to the addition of Ara C. After the completion of the treatment, the steady-state levels of MDR1 and .beta..sub.2 -microglobulin mRNA were determined by RT-PCR.

Drawing Description Paragraph Right (37):
FIG. 8B. Induction of MDR1 expression by agents that cause intracellular Ca.sup.2+ release. H9 cells were either untreated (lane 1) or treated with A23187 (10 .mu.M, lane 2) or thapsigargin (10 .mu.g/ml, lane 3) for 10 hrs at 37.degree. C., 5% CO.sub.2 in a humidified atmosphere. After the completion of the treatment, the steady-state levels of MDR1 and .beta..sub.2 -microglobulin mRNA were determined by RT-PCR.

Drawing Description Paragraph Right (38):
FIG. 8C. Effects of intracellular Ca.sup.2+ chelator on MDR1 induction by cytotoxic drugs. H9 cells were treated with Ara C(25 .mu.M shown in lanes 2 and 3, where lane 1 on the extreme left is a negative control lane receiving neither drug nor BAPTA/AM), Adriamycin (2 .mu.M; shown in lanes 4 and 5) bleomycin (100 .mu.g/ml; shown in lanes 6 and 7), 2-deoxyglucose (10 mM; shown in lanes 8 and 9) or nocodazole (2.5 .mu.M; shown in lanes 10 and 11) for 10 hrs in Ca.sup.2+ -free RPMI-1640 media/10% dialyzed fetal bovine serum as indicated. BAPTA/AM was given 45 min. prior to the addition of cytotoxic drugs. Results of assays performed in the presence of BAPTA/AM are shown in lanes 3, 5, 9 and 11. Levels of MDR1 mRNA were determined by quantitative RT-PCR.

Drawing Description Paragraph Right (39):
FIG. 8D. Effects of calmodulin inhibitor on MDR1 induction by AraC. H9 cells were either untreated (lane 1) or treated with 25 .mu.M Ara C alone (lane 2) or treated with Ara C for 10 hrs in the presence of 10 .mu.M calmidazolium chloride given 45 min. prior to Ara C (lane 3). Levels of MDR1 mRNA were determined by quantitative RT-PCR.

Drawing Description Paragraph Right (40):
FIG. 8E. Effects of inhibitors of NF-.kappa.B activation on MDR1 induction by Ara C. H9 cells were untreated (lane 1) or treated with 25 .mu.M Ara C for 10 hours (lanes 2-6) in the absence (lane 2) or in the presence of 20 .mu.M pyrrolidone dithiocarbamate (lane 3), 25 .mu.M N-tosyl-L-phenylalanine chloromethyl ketone (lane 4), 20 mM sodium salicylate (lane 5) or 10 mM aspirin (lane 6). All inhibitors were given 45 min. prior to Ara C. Quantitative RT-PCR was performed for the analysis of the levels of MDR 1 mRNA.

Drawing Description Paragraph Right (41):
FIG. 8F. Effects of inhibitors of NF-.kappa.B activation on MDR1 induction by Adriamycin. H9 cells were treated under the same conditions as in FIG. 8E using Adriamycin (2 .mu.M) instead of Ara C.

Drawing Description Paragraph Right (42):
FIG. 9. cDNA-PCR analysis of inhibition by protein kinase inhibitors of MRP mRNA expression. In each lane, the upper band (292 bp) corresponds to MRP, and the lower band (120 bp) to .beta..sub.2 -microglobulin specific PCR products, amplified in separate tubes.

Drawing Description Paragraph Right (43):
FIG. 9A. MRP mRNA expression inhibition in drug-treated H-9 cells. Cells were incubated for 10 hours with 30 nM staurosporine (Lanes 3 and 4), 5 .mu.M chelerythrine (Lanes 5 and 6), 32 .mu.M methyl-2,5-dihydroxycinnamate (erbstatin analog; Lanes 7 and 8) or 10 mM neomycin sulfate (Lanes 9 and 10). Negative control lanes (Lanes 1 and 2) represent

H-9 cell cultures incubated overnight in the absence of any drug.

Detailed Description Paragraph Right (1):
The present invention relates to the use of particular inhibitors of intracellular signal transduction in eukaryotic, particularly mammalian, cells, to prevent the emergence of the multidrug resistance phenotype in cancer cells. The discovery of MDR1 induction by cytotoxic drugs and the ability of the inhibitors disclosed herein to prevent such induction are fully described and exemplified in the Examples below. For clarity of discussion, the invention is described in terms of a variety of signal transduction inhibitors, including protein kinase inhibitors, cytoplasmic calcium antagonists and calmodulin inhibitors, phosphoinositol-dependent phospholipase C inhibitors, and inhibitors of transcription factor NF-.kappa.B activation, which have potent effects, for example, on PKC activity in a panel of human tumor cell lines. However, the invention can be analogously applied to a wide variety of in vitro cell lines and in vivo tumors treated with other chemotherapeutic drugs, using any of the inhibitors of the invention, as well as analogues and derivatives thereof and additional compounds not explicitly disclosed herein which act at the same or equivalent signal transduction targets. Specific embodiments of certain inhibitors are exemplified as described below. It will be understood that any of a variety of analogues of the specific inhibitors disclosed herein, known in the art or prepared using methods known in the art, are also encompassed by the invention as herein described.

Detailed Description Paragraph Right (2):
TPA (12-o-tetradecanoylphorbol-13-acetate), an efficient PKC activator, and diacylglycerol, a physiological stimulant of PKC, are shown in Examples 1 and 2, below, to increase MDR1 gene expression in normal human PBL, and in cell lines derived from different types of leukemias or solid tumors. The effect of TPA is observed in all the tested cell lines that expressed P-glycoprotein prior to treatment, and in some but not all other cell lines without detectable P-glycoprotein expression prior to treatment. It is possible, however, that MDR1 expression could be induced in the non-responsive cell lines by higher concentrations of TPA than those tested as described herein. The observed effects of TPA and diacylglycerol indicate that MDR1 expression in human cells may be regulated through a PKC-mediated signal transduction pathway.

Detailed Description Paragraph Right (3):
The increase in MDR1 expression in cells treated with the PKC agonists is observed at the level of both P-glycoprotein and steady-state levels of MDR1 mRNA, which may reflect either increased transcription or decreased mRNA degradation. It is known that the major ("downstream") promotor of the human MDR1 gene (Ueda et al., 1987, J. Biol. Chem. 262: 505-508) contains an AP-1 site responsible for the stimulation of transcription by TPA (Angel et al., 1987, Cell 49: 729-739; Lee et al., 1987, Cell 49: 741-752). The AP-1 site and its surrounding sequences are conserved between the human MDR1 gene and its rodent homologs (Hsu et al., 1990, Molec. Cell Biol. 10: 3596-3606; Teeter et al., 1991, Cell Growth Diff. 2: 429-437). The AP-1 sequence of the hamster pgp1 gene was shown to be an essential positive regulator of its promoter (Teeter et al., 1991, Cell Growth Diff. 2: 429-437), although the corresponding element of the homologous mouse mdrla (mdr3) gene may have a negative regulatory effect (Ikeguchi et al., 1991, DNA Cell Biol. 10: 639-649). Thus, it is possible that the AP-1 element of the human MDR1 promoter is directly responsible for the stimulation of MDR1 expression by PKC agonists.

Detailed Description Paragraph Right (4):
The induction of MDR1 gene expression by a PKC-mediated pathway is consistent with previous observations that multidrug-resistant cell lines selected for increased P-glycoprotein expression frequently contained elevated levels of PKC (Aquino et al., ibid.; Fine et al., ibid.; O'Brian et al., ibid.; Posada et al., ibid.). An increase in PKC activity could represent an early event responsible for increased MDR1 gene expression during the selection of such cell lines. This interpretation does not preclude, however, that the phosphorylation of the induced P-glycoprotein by PKC could further increase P-glycoprotein activity. Evidence for the latter hypothesis comes from the study of Yu et al. (1991, Cancer Commun. 3: 181-189), who found that the level of drug resistance in a multidrug-resistant subline of MCF-7 cells, obtained after transfection with MDR1 cDNA transcribed from a heterologous promoter, could be increased by the introduction of a vector expressing high levels of PKC.alpha.. The increased resistance in the PKC.alpha. transfectants was accompanied by increased P-glycoprotein phosphorylation, without apparent changes in expression levels.

Detailed Description Paragraph Right (5):

PKC plays a central role in various signal transduction pathways, associated with different adaptive, proliferative and differentiative processes. Even though PKC agonists have been found to induce MDR1 expression in normal and malignant hematopoietic cells, the same result has not been achieved using hematopoietic growth factors that may also act through PKC-mediated pathways. Furthermore, PKC agonists induce MDR1 expression in cell lines of not only hematopoietic but also epithelial origin, indicating that PKC-mediated regulation of MDR1 expression may have a general physiological role.

Detailed Description Paragraph Right (6):
PKC-mediated mechanisms have been implicated in the transcriptional response to DNA damage by UV irradiation or alkylating agents (Kaina et al., 1989, in M. W. Lambert and J. Laval (ed.), DNA Repair Mechanisms and Their Biological Implications in Mammalian Cells, Plenum Press, New York; Papathanasiou and Fornace, 1991, pp. 13-36 in R. F. Ozols (ed.), Molecular and Clinical Advances in Anticancer Drug Resistance, Kluwer Academic Publishers, Boston, Mass.). PKC activation has also been associated with cellular responses to other cytotoxic drugs, such as cytosine arabinoside (Kharbanda et al., 1991, Biochemistry Cancer Res. 49: 6634-6639). Thus, PKC-mediated induction of MDR1 expression could be a part of a general stress response to different types of cellular damage, including the damage produced by cytotoxic chemotherapeutic drugs.

Detailed Description Paragraph Right (7):
The present invention discloses that MDR1 expression in human leukemia and solid-tumor derived cell lines can be induced by short-term exposure to a number of different cytotoxic drugs that are used in cancer chemotherapy (see Example 3, below). MDR1 induction, both at the mRNA and at the protein levels, was observed in a subpopulation of cells treated with either P-glycoprotein-transported agents (Adriamycin, daunorubicin, vinblastine, etoposide) or chemotherapeutic drugs that are not transported by P-glycoprotein (such as methotrexate, 5-fluorouracil, chlorambucil, cisplatinum, hydroxyurea and 1-.beta.-D-arabinofuranosylcytosine (Ara-C), an effective anti-leukemic drug (Kharbanda et al., 1991, Biochemistry 30: 7947-7952)). Since MDR1 expression does not provide resistance to drugs of the second group, and because MDR1 induction could be achieved after short times of drug exposure (less than one cell generation in many cases), these findings indicate that cytotoxic selection for MDR1-expressing cells was not responsible for the observed increase in MDR1 expression. MDR1 induction became detectable at the same time as visible cell damage, indicating that expression was more likely to be an indirect consequence of such damage, rather than a direct response to specific agents.

Detailed Description Paragraph Right (8):
Most importantly, MDR1 expression induced by treatment with cytotoxic drugs did not disappear after the removal of the drug, but was maintained for at least several weeks in cells cultured in drug-free media. P-glycoprotein-positive cells, growing in the absence of the drugs, showed no change in differentiation state. This result indicates that drug-induced MDR1 expression is a stable phenomenon which is not limited to dying or terminally differentiating cells. In addition to increased MDR1 expression, drug-treated cells displayed a 2-3 fold increase in resistance to vinblastine, a P-glycoprotein transported drug; such resistance was specifically associated with MDR1-expressing cells. Drug-treated cells also showed increased resistance to chlorambucil, a chemotherapeutic drug which is not transported by P-glycoprotein. The latter finding suggests that some other clinically relevant mechanisms of drug resistance may be co-induced with MDR1 expression after treatment with cytotoxic drugs.

Detailed Description Paragraph Right (9):
Taken together, these findings suggested that treatment of human tumor cells with various drugs used in cancer chemotherapy could induce MDR1 expression directly, rather than by selection of preexisting genetic variants, as previously believed. The resulting increase in multidrug resistance was found to be stable and could be sufficient to reduce the response to chemotherapeutic drugs both in vitro and in vivo. It is likely that drug-mediated induction of MDR1 expression could occur during cancer chemotherapy, and could account, at least in part, for the observed increase in the incidence of MDR1 expression in drug-treated human tumors. This invention therefore provides the first demonstration of MDR1 induction under clinically relevant conditions and suggests that PKC may play a central role in such induction. This provides a basis for chemotherapeutic protocols that would prevent MDR1 induction during cancer chemotherapy through the inhibition of PKC.

Detailed Description Paragraph Right (10):

The present invention demonstrates that protein kinase inhibitors, especially those with potent activity against PKC, are capable of preventing the induction of MDR1 gene expression in cancer cells. For example, staurosporine, a potent but non-selective inhibitor of PKC (Ruegg and Burgess, 1989, Trends Pharmacol. Sci. 10: 218-220), was found (at a concentration of 30 nM) to prevent MDR1 induction in P-glycoprotein-negative cells treated with TPA, diacylglycerol and a number of chemotherapeutic cytotoxic drugs, including Ara-C, vinblastine, methotrexate and Adriamycin. A variety of other protein kinase inhibitors, including H7 (50 .mu.M), calphostin C (at a concentration of 1 .mu.M, used in conjunction with white light illumination), and chelerythrine (5 .mu.M), are also shown to prevent MDR1 induction by chemotherapeutic drugs.

Detailed Description Paragraph Right (11):
Inhibition of MDR1 expression induced by treatment with Ara-C has also been demonstrated using inhibitors that have not previously been understood to have specificity for protein kinase C. Examples of such inhibitors include erbstatin and analogues of erbstatin, and neomycin sulfate. Erbstatin and its analogues, such as methyl-2,5-dihydroxycinnamate, are known to be capable of inhibiting the epidermal growth factor receptor tyrosine kinase (Umezawa and Imoto, 1991, Meth. Enzymol. 201: 379-385). This erbstatin analog was found to completely inhibit Ara-C induced MDR1 expression at a concentration of 32 .mu.M (see, Example 4, below). This erbstatin analog was also found to inhibit MDR1 expression induced by treatment of sensitive cells with TPA, a known PKC agonist, consistent with anti-PKC activity (see Example 4 and FIG. 7F, infra). In addition to the erbstatin analogue, two other compounds known as inhibitors of protein tyrosine kinase, tyrphostin A25 and tyrphostin B46, were also found to inhibit Ara-C induced MDR1 expression, at concentrations of 50-100 .mu.M.

Detailed Description Paragraph Right (12):
The antibacterial compound, neomycin sulfate, was found to inhibit Ara-C induced MDR1 expression, when contacted with induced cells at a concentration of 10 mM. This concentration of neomycin showed no cellular toxicity on human H9 cells, and appears to be within the range of clinically-achievable concentrations. This compound has the advantage of being of general benefit to patients undergoing chemotherapy, and has been widely used for the treatment of bacterial intestinal infections in humans.

Detailed Description Paragraph Right (14):
These findings provide evidence that protein kinase inhibitors, and in particular PKC inhibitors, are involved in MDR1 induction and suggest the possibility of using protein kinase inhibitors to prevent MDR1 gene activation.

Detailed Description Paragraph Right (15):
Other protein kinase inhibitors, however, were found to be ineffective in inhibiting Ara-C induced MDR1 expression. These include the PKC inhibitor D,L-threo-sphingosine (when used at 5 .mu.M concentration; higher concentrations induced cellular toxicity). This was the only PKC inhibitor tested that did not inhibit MDR1 induction, suggesting that the observed cellular toxicity prevented administration of this compound at an effective inhibitory concentration. The tyrosine kinase inhibitor, herbimycin A, was also found to be ineffective at inhibiting MDR1 induction by Ara-C at concentrations ranging from 0.35-3 .mu.M. Another tyrosine kinase inhibitor, genistein, only weakly inhibited Ara-C induced MDR1 expression, at a concentration of 150 .mu.M.

Detailed Description Paragraph Right (16):
Staurosporine is a P-glycoprotein inhibitor that may bind directly to P-glycoprotein (Sato et al., 1990, Biochem. Biophys. Res. Commun. 173: 1252-1257). In some P-glycoprotein-positive cell lines, however, staurosporine, when used alone, significantly increased P-glycoprotein expression. Additionally, two other P-glycoprotein binding compounds, cyclosporine A and verapamil, also known PKC inhibitors, increased P-glycoprotein expression in some of the P-glycoprotein-positive cell lines. These results suggest that protein kinase inhibitors may be more effectively used to prevent an increase in MDR1 in P-glycoprotein-negative or nearly negative tumors than in tumors already expressing P-glycoprotein in a large fraction of tumor cells. It should be noted, however, that the finding that staurosporine increased P-glycoprotein expression in a small number of hematopoietic that augmentation of P-glycoprotein expression by PKC inhibitors is a general property of P-glycoprotein-positive tumor cells, and that patients with P-glycoprotein-positive tumors cannot benefit from the use of protein kinase inhibitors to prevent further drug-induced increase of multidrug resistance in tumor cells.

Detailed Description Paragraph Right (17):

P-glycoprotein negative solid tumors or leukemias can be identified by the analysis of biopsy material, surgical or hematological specimens of patients'tumors using techniques well known in the art (Roninson, ibid.). These techniques include but are not limited to immunocytochemical, immunohistochemical or immunofluorescent assays with P-glycoprotein-specific antibodies; vital staining with P-glycoprotein transported fluorescent dyes; Northern blot, dot blot or slot blot hybridization with MDR1-specific nucleic acid probes; or cDNA-PCR analysis of MDR1 mRNA. Working examples of some of the above assays are described in Examples 1 and 3, below. It should be noted that some cell lines that appear to be P-glycoprotein negative by protein or function-based assays are described in levels of MDR1 mRNA when assayed by cDNA-PCR (see Table I). This indicates that protein or function-based assays would be preferable as the primary criterion for the identification of tumors that are likely to benefit from the use of protein kinase inhibitors. Alternatively, cDNA-PCR or other methods for MDR1 mRNA measurement may be used with the understanding that MDR1 mRNA expression at the level of K562 cells or slightly (e.g. 2-fold) higher may still be indicative of P-glycoprotein negative tumors.

Detailed Description Paragraph Right (18):
Although the protein kinase inhibitors tested herein are known to be non-selective in their inhibitory activities, i.e., their action is not specific for PKC, the studies described herein provide evidence that their ability to inhibit PKC activity may be a critical factor in the prevention of MDR1 induction. For example, a number of potent PKC inhibitors, including staurosporine, H7, chelerythrine, neomycin sulfate and calphostin C, are capable of inhibiting MDR1 induction by cytotoxic drugs. In contrast, HA1004, a protein kinase inhibitor that is inactive against PKC, is shown to be totally ineffective in preventing MDR1 induction. Hence, it is likely that any protein kinase inhibitor that is capable of inhibiting PKC, irrespective of its specificity for PKC, would be useful in preventing MDR1 induction in cancer cells.

Detailed Description Paragraph Right (19):
Accordingly, any protein kinase inhibitor capable of preventing the induction of MDR1 by chemotherapeutic drugs as measured by any method described in Example 3, below, such as fluorescent dye accumulation, cDNA-PCR for MDR1 mRNA or staining with P-glycoprotein specific antibody, may be used in the practice of the method of the invention. Such inhibitors may be administered in a cancer patient bearing a solid tumor or leukemia prior to and/or simultaneously with treatment by chemotherapeutic drugs. Any anti-cancer drug commonly used in cancer chemotherapy is encompassed within the scope of this regimen, including, but not limited to, Ara-C, Adriamycin, daunorubicin, vinblastine, etoposide, methotrexate, 5-fluorouracil, chlorambucil, cisplatin, and hydroxyurea.

Detailed Description Paragraph Right (20):
Prior to the present invention, a number of compounds capable of inhibiting PKC have been investigated in vitro and in vivo for potential use in cancer chemotherapy. However, it should be noted that while such compounds were found to show selective growth inhibition for tumor relative to normal cells (Powis and Kozikowski, 1991, Clin. Biochem. 24: 385-397; Grunicke et al., 1989, Adv. Enzyme Regul. 28: 201-216) they have not been shown or suggested to be capable of preventing MDR1 expression in cancer cells. In vitro studies have shown that the anti-proliferative effects of PKC inhibitors occurred at approximately the same dose as their PKC inhibitory activity (Grunicke et al., ibid.). Compounds tested in vivo include staurosporine and its benzoyl derivative CGP 41 251, which were found in nude mice to show anti-tumor effect at one-tenth of their maximum tolerated doses (MTD) (MTD was 1 mg/kg for staurosporine and 250 mg/kg for CGP 41 251) (Meyer et al., 1989, Int. J. Cancer 43: 851-856). Other staurosporine analogs shown to have antitumor activity in vivo include UCN-01 (Takahashi et al., 1987, J. Antibiot. 40: 1782-1784) and 8-N-(diethylaminoethyl) rebeccamycin (BMY 27557) (Schurig et al., 1990, Proc. Amer. Assoc. Cancer Res. 31: Abs. 2469). For the latter compound, optimal doses for i.p. administration ranged from a total of 108 mg/kg administered over nine injections/day at 12 mg/kg/injection to a single dose of 64 mg/kg.

Detailed Description Paragraph Right (23):
The epidermal growth factor receptor tyrosine kinase inhibitor erbstatin has been reported to have antitumor activity in vivo (Imoto et al., 1987, Japanese J. Cancer Res. 78: 329-332; Toi et al., 1990, Eur. J. Cancer 26: 722-724). No evidence has been reported that erbstatin has any capacity to inhibit MDR1 induction by cytotoxic drugs.

Detailed Description Paragraph Right (24):
While none of the above compounds (with the exception of staurosporine described in

Examples 2 and 4, below) have been tested for the ability to prevent MDR1 induction by cytotoxic drugs, the results disclosed in the present invention strongly indicate that they are likely to possess such an effect, since all of them are capable of inhibiting PKC. The availability of in vivo animal and clinical trial data for these and other PKC inhibitors enables those skilled in the art to use such compounds in combination with conventional anticancer drugs to prevent the emergence of multidrug resistance during chemotherapy. These compounds may be administered with chemotherapeutic drug treatment at a dose range of about 1-250 mg/kg body weight, either by repeated injections, by continuous infusion, or as topical treatment.

Detailed Description Paragraph Right (25):
In addition to these aspects of the present invention, an in vitro assay is disclosed for rapid identification of any compound which is capable of preventing the induction of MDR1 gene expression by chemotherapeutic drugs. For example, H-9 or K562 leukemia cell lines are treated with a test compound for about 30 minutes for 10-36 hours (though any time of culture over 1 hour may be sufficient, see FIG. 2B) prior to exposure to 10-25 .mu.M Ara-C or 200 ng/mL vinblastine under standard tissue culture conditions, followed by evaluation of MDR1 induction by the drugs in cultures treated with the test compound, as compared to controls. Test compounds identified by such assays as being capable of preventing MDR1 induction by chemotherapeutic drugs, are used for patient treatment in the same manner as protein kinase inhibitors described herein.

Detailed Description Paragraph Right (26):
The present invention also demonstrates that inhibitors of phosphoinositol-dependent phospholipase C (PI-PLC) are capable of preventing induction of MDR1 gene expression in cancer cells. The antibiotic neomycin sulfate, an inhibitor of PI-PLC, was found to completely prevent cytotoxic drug and PKC agonist induction of MDR1 expression at a concentration of 10 mM (see FIG. 8A). The present invention discloses the use of another inhibitor of PI-PLC,
1-(6-((1.beta.-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl)-1-H-pyrro le-2,5-dione (U73122; see Heemskerek et al., 1994, Eur. J. Biochem.223: 543-551), that reduced the steady-state levels of MDR1 mRNA in Ara C-treated H9 cells. U73122 was active at significantly lower concentrations (10 .mu.M) than neomycin sulfate.

Detailed Description Paragraph Right (27):
In contrast, the inhibitor of phosphatidylcholine-specific phospholipase C (PC-PLC), D609 (tricyclodecan-9-yl-xanthogenate potassium) was without an effect when added at concentrations up to 100 .mu.g/ml, the dose which has been shown to inhibit PC-PLC (see Wiegmann et al., 1994, Cell 78: 1005-1015). The high activity and specificity of U73122, including analogues and derivatives thereof that are specific inhibitors of PI-PLC, are advantageous for use in combined regimens of chemotherapeutic drug treatment to prevent the induction of MDR1 mediated drug resistance in cancer cells.

Detailed Description Paragraph Right (28):
These results indicate that any inhibitor of PI-PLC may be used to prevent induction of MDR1 gene expression by cytotoxic, particularly chemotherapeutic, drugs. PI-PLC inhibitors capable of preventing the induction of MDR1 by chemotherapeutic drugs as measured by any method described in Example 3, below, such as fluorescent dye accumulation, cDNA-PCR for MDR1 mRNA or staining with P-glycoprotein specific antibody, may be used in the practice of the method of the invention. Such inhibitors may be administered in a cancer patient bearing a solid tumor or leukemia prior to and/or simultaneously with treatment by chemotherapeutic drugs. Any anti-cancer drug commonly used in cancer chemotherapy is encompassed within the scope of this regimen, including, but not limited to, Ara-C, Adriamycin, daunorubicin, vinblastine, etoposide, methotrexate, 5-fluorouracil, chlorambucil, cisplatin, and hydroxyurea.

Detailed Description Paragraph Right (29):
The present invention also demonstrates that cytoplasmic calcium antagonists and calmodulin inhibitors are capable of preventing induction of MDR1 gene expression in cancer cells. As used herein, the term "cytoplasmic calcium antagonist" will be understood to encompass calcium-specific chelators, agents which inhibit the intracellular release of calcium ions from cytoplasmic stores, and any agent which inhibits an increase in free cytoplasmic calcium concentration.

Detailed Description Paragraph Right (30):
Two types of agents which increase the level of intracellular Ca.sup.2+, namely the specific Ca.sup.2+ ionophore A23187 (see Geng et al., 1995, J. Cell Biol. 129: 1651-1657) and an inhibitor of Ca.sup.2+ -dependent ATPase, thapsigargin (see

Chakrabarti et al., J. Cell. Biochem. 58: 344-359) are capable of inducing MDR1 gene expression in H9 cells (FIG. 8B). A highly-specific Ca.sup.2+ ion chelator, 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid, tetra(acetoxymethyl)ester (BAPTA/AM; see Bunn et. al., 1995, J. Neurochem. 64: 1370-1378) was found to inhibit induction of MDR1 gene expression by cytotoxic drugs. Pretreatment of H9 cells with BAPTA/AM at concentrations as low as 5 .mu.M were found to completely abrogate the induction of MDR1 expression by different cytotoxic agents, such as Ara C(25 .mu.M), Adriamycin (2 .mu.M), bleomycin (100 .mu.g/ml), 2-deoxyglucose (10 mM) and nocodazole (2.5 .mu.M) (FIG. 8C). This inhibitory effect of BAPTA/AM was detectable when the chelator was given to the cells within the first 3 hrs prior to the addition of MDR1 inducing agents.

Detailed Description Paragraph Right (31):
An inhibitor of intracellular release of calcium ions from cytoplasmic stores, 8-(dimethylamino)octyl-3,4,5-trimethoxybenzoate hydrochloride (TMB-8; Gandhi et al., 1992, Biochim. Biophys. Acta 1136: 68-74), was found to completely abrogate the ability of AraC to induce MDR1 gene expression in H9 cells when present at a concentration of 200 .mu.M.

Detailed Description Paragraph Right (32):
In addition, a calmodulin inhibitor (calmidazolium chloride; see Silver et al., 1986, Biochem. Pharmacol. 35: 2545-2551) was found to inhibit AraC-induced MDR1 gene expression when H9 cells were treated with the inhibitor at a concentration of 10 .mu.M (see FIG. 8D).

Detailed Description Paragraph Right (33):
These results indicate that cytoplasmic calcium antagonists and calmodulin inhibitors may be used to prevent induction of MDR1 gene expression by cytotoxic, particularly chemotherapeutic, drugs. Such inhibitors include calcium ion-specific chelators, calmodulin inhibitors and inhibitors of the intracellular effectors of calcium ion release, particularly from intracellular calcium stores such as endoplasmic reticulum. Inhibitors capable of preventing the induction of MDR1 by chemotherapeutic drugs as measured by any method described in Example 3, below, such as fluorescent dye accumulation, cDNA-PCR for MDR1 mRNA or staining with P-glycoprotein specific antibody, may be used in the practice of the method of the invention. Such inhibitors may be administered in a cancer patient bearing a solid tumor or leukemia prior to and/or simultaneously with treatment by chemotherapeutic drugs. Any anti-cancer drug commonly used in cancer chemotherapy is encompassed within the scope of this regimen, including, but not limited to, Ara-C, Adriamycin, daunorubicin, vinblastine, etoposide, methotrexate, 5-fluorouracil, chlorambucil, cisplatin, and hydroxyurea.

Detailed Description Paragraph Right (34):
The present invention also demonstrates that inhibitors of activation of eukaryotic transcription factor NF-.kappa.B are capable of preventing induction of MDR1 gene expression in cancer cells. It has been shown that AraC treatment of mammalian cells results in release of transcription factor NF-.kappa.B from an inhibitory subunit, followed by translocation of the active NF-.kappa.B to the nucleus (see Siebenlist et al., 1994, Annu. Rev. Cell Biol. 10: 405-455; Kopp et al., 1994, Science 265: 956-959). A variety of agents known to inhibit the activation of NF-.kappa.B as the result of stress stimuli were found to prevent induction of MDR1 gene expression by chemotherapeutic drugs. Pretreatment of H9 cells with pyrrolidone dithiocarbamate (100 .mu.M), N-tosyl-L-phenylalanine chloromethyl ketone (25 .mu.M), sodium salicylate (20 mM) or aspirin (acetylsalicylic acid, 10 mM) was found to completely abrogate induction of MDR1 gene expression by either Ara C (25 .mu.M) or Adriamycin (2 .mu.M).

Detailed Description Paragraph Right (35):
These results indicate that inhibitors of NF-.kappa.B activation may be used to prevent induction of MDR1 gene expression by cytotoxic, particularly chemotherapeutic, drugs. Such inhibitors include aspirin, a commonly-used analgesic with a long record of accepted clinical use. This compound is now understood as being advantageous to patients undergoing cancer chemotherapy by preventing the induction of resistance to chemotherapeutic drugs.

Detailed Description Paragraph Right (36):
NF-.kappa.B activation inhibitors capable of preventing the induction of MDR1 by chemotherapeutic drugs as measured by any method described in Example 3, below, such as fluorescent dye accumulation, cDNA-PCR for MDR1 mRNA or staining with P-glycoprotein specific antibody, may be used in the practice of the method of the invention. Such inhibitors may be administered in a cancer patient bearing a solid tumor or leukemia

prior to and/or simultaneously with treatment by chemotherapeutic drugs. Any anti-cancer drug commonly used in cancer chemotherapy is encompassed within the scope of this regimen, including, but not limited to, Ara-C, Adriamycin, daunorubicin, vinblastine, etoposide, methotrexate, 5-fluorouracil, chlorambucil, cisplatin, and hydroxyurea.

Detailed Description Paragraph Right (37):
The present invention demonstrates that protein kinase inhibitors are also capable of inhibiting expression of a protein, termed the multidrug resistance-associated protein (MRP; Grant et al., ibid.). In contrast to MDR1, certain human tumor cells, such as H9 human T-cell leukemia cells, display robust expression of the MRP gene, as evidenced by cDNA-PCR experiments as disclosed in Example 8 and FIG. 9A, below. Treatment of such cells with certain protein kinase inhibitors results in a dramatic reduction of MRP expression. Compounds shown to be capable of inhibiting MRP expression in H-9 cells include staurosporine (at a concentration of 100 nM), neomycin sulfate (10 mM), chelerythrine (1-5 .mu.M), and the erbstatin analogue, methyl-2,5-dihydroxycinnamate (32 .mu.M).

Detailed Description Paragraph Right (38):
The present invention thus provides a number of agents having related activities of benefit to cancer chemotherapy patients. These agents prevent the activation of the MDR1 gene by chemotherapeutic drugs, thereby suppressing the emergence of MDR1-mediated multidrug resistance during chemotherapy of patients bearing MDR1-negative tumors. In addition, these agents reduce MRP expression, thereby decreasing multidrug resistance mediated by MRP in MRP-positive tumors. One of these agents, neomycin sulfate, has the additional advantage of being a commonly-used antibiotic, which has been well-characterized clinically and whose use may have added benefits for combating adventitious bacterial infections in cancer chemotherapy patients.

Detailed Description Paragraph Right (48):
RNA was extracted from approximately 10.sup.6 cells by a small-scale sodium dodecyl sulfate extraction procedure (Peppel and Baglioni, 1990, BioTechniques 9: 711-713). In the alternative, RNA was isolated from cells using the TRIzol method (commercially available from GIBCO/BRL). cDNA synthesis and polymerase chain reaction (PCR) amplification of MDR1 and .beta..sub.2 -microglobulin cDNA sequences were carried out essentially as described (Noonan et al., 1990, Proc. Natl. Acad. Sci. USA 87: 7160-7164; Noonan and Roninson, 1991, in Roninson (ed.), Molecular and Cellular Biology of Multidrug Resistance in Tumor Cells, Plenum Press, New York, pp. 319-333). Briefly, cDNA was prepared using the method of Krug and Berger (1987, Meth. Enzymol. 152: 316-324), with the modification that random hexamers were substituted for oligo(dT) as primers for cDNA synthesis. (Commercially-available kits (for example, from GIBCO/BRL, Gaithersburg, Md.) can be advantageously modified for making cDNA using random hexamers.) PCR is then performed using the following primers:

Detailed Description Paragraph Right (50):
To determine if the observed effect of PKC stimulants on P-glycoprotein activity was due to an increase in P-glycoprotein expression, untreated and TPA-treated PBL were stained by indirect immunofluorescence labeling with mAb UIC2 that recognized an extracellular epitope of P-glycoprotein encoded by the human MDR1 gene. TPA treatment markedly increased the levels of P-glycoprotein on the cell surface. The increase in P-glycoprotein was accompanied by a corresponding increase in MDR1 mRNA levels in the total population of TPA-treated PBL, as detected by polymerase chain reaction (PCR) amplification of MDR1 cDNA sequence. Thus, the TPA-induced increase in P-glycoprotein activity was due at least in part to the activation of MDR1 gene expression at the mRNA and protein levels.

Detailed Description Paragraph Right (52):
Among the cell lines that expressed no detectable P-glycoprotein, H9 and K562 leukemia cell lines showed clear-cut induction of MDR1 mRNA and P-glycoprotein by either TPA or DOG. Flow cytometric assays showed that the treatment of these cell lines with TPA or DOG resulted in the appearance of a major cell population that expressed P-glycoprotein (FIGS. 1A through 1E). These changes were paralleled by an increase in steady-state levels of MDR1 mRNA in TPA- or DOG-treated cells (FIGS. 2A and 2B). As shown in FIG. 2B, MDR1 mRNA became detectable in H9 cells 2 hrs after the addition of TPA and continued to increase until at least 5 hr, indicating a rapid response to TPA, consistent with transcriptional activation of MDR1 by TPA in these cells.

Detailed Description Paragraph Right (53):
The increase of MDR1 expression after TPA treatment was not limited to hematopoietic

cells, but was also observed in some solid tumor-derived cell lines, including EJ bladder carcinoma cells that expressed a low level of P-glycoprotein, and MCF-7 breast carcinoma cells where MDR1 expression was undetectable without TPA treatment (FIG. 2C). As summarized in Table I, most of the tested P-glycoprotein negative cell lines were only treated with a fixed concentration (20 ng/mL) of TPA, and were not tested for their ability to respond to higher TPA concentrations.

Detailed Description Paragraph Right (54):
In an attempt to interfere with the induction of MDR1 gene expression by PKC agonists, a potent protein kinase inhibitor, staurosporine, was used to treat various cell lines. Unexpectedly, staurosporine alone caused a significant increase in P-glycoprotein expression in the cell lines that were already positive for P-glycoprotein (KG1, KG1a, mouse EL4 and LBRM 33 cell lines). Two additional compounds, cyclosporine A and verapamil, which are known to be P-glycoprotein inhibitors as well as inhibitors of PKC, have also been found to increase P-glycoprotein expression and/or dye efflux in KG1 and EL4 cells. The effect of PKC inhibitors on P-glycoprotein expression in the P-glycoprotein-positive cell lines made it difficult to analyze the interactions between staurosporine and PKC agonists in such cells.

Detailed Description Paragraph Right (59):
1.3. MDR1 Induction Inhibition Assays using PKC Inhibitors

Detailed Description Paragraph Right (60):
Cells were plated in 6-well Falcon tissue culture plates at 3,300 cells per well, and incubated in the appropriate concentrations of drugs. Each of the protein kinase inhibitors to be tested were added at the appropriate final concentration 45 minutes prior to MDR1 induction by the addition of Ara-C or TPA, with the exception of herbimycin A, which was added 12 hours prior to Ara-C or TPA, and tyrphostins A25 and B46, which were each added 16 hours prior to MDR1 induction by the addition of Ara-C or TPA. Cells were incubated overnight (TPA) or for 10 hours (Ara-C) at 37.degree. C./ 5% CO.sub.2 after the addition of the MDR1 inducing agents before harvesting for cDNA-PCR analysis.

Detailed Description Paragraph Right (64):
The studies described in Examples 1 and 2, above, demonstrate that PKC agonists can induce MDR1 expression, suggesting an important role in PKC in the activation of the multidrug resistance response in tumor cells. PKC has also been implicated in cellular responses to different types of cytotoxic stress (Papathanasiou and Fornace, 1991, in R. F. Ozols (ed.), Molecular and Clinical Advances in Anticancer Drug Resistance, Kluwer Academic Publishers, Boston, Mass., pp. 13-36). In particular PKC is activated by treatment with Ara-C. Therefore, experiments were performed to test whether Ara-C, which is not transported by P-glycoprotein, would have any effect on P-glycoprotein function in K562 leukemia cells. As illustrated in FIG. 3A, exposure to K562 cells to Ara-C for 12-72 hours led to the emergence of a subpopulation of 3-17% cells that effluxed Rh123. Rh123 efflux was sensitive to the P-glycoprotein inhibitor verapamil. The appearance of Rh123-dull cells was paralleled by a dose-dependent increase in MDR1 mRNA expression relative to .beta..sub.2 -glycoprotein in Ara-C treated K562 cells, as detected by polymerase chain reaction amplification of cDNA sequences (FIG. 4A).

Detailed Description Paragraph Right (65):
A number of other chemotherapeutic drugs were also tested for their ability to induce MDR1 expression in K562 cells. Adriamycin, daunorubicin, vinblastine, cisplatin and hydroxyurea were all found to induce MDR1 mRNA expression (FIG. 4B) and Rh123 or DiOC.sub.2 (3) efflux by 3-10% of the treated cells (FIG. 3A). Only the first four of these drugs are transported by P-glycoprotein (Roninson, ibid.). This result, together with the short times of drug exposure required for MDR1 induction, indicates that cytotoxic selection for MDR1-expressing cells was not responsible for the emergence of the P-glycoprotein-positive subpopulations.

Detailed Description Paragraph Right (66):
The ability of cytotoxic drugs to induce MDR1 expression was not limited to K562 cells. Ara-C increased P-glycoprotein expression in KG1 leukemia cells, which contained a significant amount of P-glycoprotein prior to drug treatment, as detected by Rh123 accumulation or immunoreactivity with monoclonal antibody UIC2 (FIG. 3A). Ara-C also activated MDR1 mRNA expression in H9 T-cell leukemia (FIGS. 5A through 5C), KB-3-1 epidermoid carcinoma (FIG. 4C), and EJ bladder carcinoma (FIG. 4D), though the magnitude of induction was somewhat lower in carcinoma cell lines. In addition, MDR1 mRNA expression was induced in H9 cells by treatment with Adriamycin, vinblastine and methotrexate (FIGS. 5A through 5C), and in KB-3-1 cells with Adriamycin (FIG. 4C).

However, P-glycoprotein induction was not detected in HL60 leukemia cells treated with these drugs. In all cases, MDR1 induction°became detectable at the same time as visible cell damage, which was evidenced by cell swelling, increased granularity, altered cell shape, and growth inhibition (FIG. 4A). In addition, continuous passage of some cell lines in the absence of drugs for several months also led to a small increase in MDR1 expression, accounting for the variability in the basal levels of MDR1 mRNA in untreated cells.

Detailed Description Paragraph Right (67):
Also assayed was whether drug-induced MDR1 expression was maintained after cytotoxic drug treatment. For this purpose, K562 cells were treated with cytotoxic concentrations of Ara-C, Adriamycin, chlorambucil or methotrexate for 3-5 days, and then allowed to grow in the absence of the drugs. At different times, MDR1 expression in the surviving cells was analyzed by dye efflux and immunofluorescence labeling with UIC2 (FIG. 3C) or by cDNA-PCR (FIG. 4E). MDR1 expression in a subpopulation of treated cells was maintained for a least several weeks after the removal of drug (up to 11 weeks in the Ara-C treated population). P-glycoprotein-positive K562 cells showed no significant changes in their size, granularity and expression of differentiation-related antigenic markers. The presence of multidrug-resistant cells six weeks after the removal of Ara-C or Adriamycin was also demonstrated by a growth inhibition assay with vinblastine, a P-glycoprotein substrate. Vinblastine resistance, characterized by approximately 2-3 fold increase in the ID.sub.10 value, was specifically associated with the Rh123-dull subpopulation of cells (FIGS. 6A and 6B). Thus, drug treatment leads to sustained induction of MDR1 expression and its associated drug resistance in a subpopulation of treated cells. It was also found that Ara-C and Adriamycin-treated K562 cells were more resistant than the untreated cells to the cytotoxic effect of chlorambucil, a chemotherapeutic alkylating agent which is not transported by P-glycoprotein. This result indicates that other pathways or mechanism of clinically relevant drug resistance are co-induced with MDR1 expression after treatment with chemotherapeutic drugs.

Detailed Description Paragraph Right (68):
To demonstrate that PKC was involved in MDR1 induction by cytotoxic drugs, a variety of PKC inhibitors were used to block MDR1 mRNA induction in H9 cells. The addition of certain these compounds effectively blocked MDR1 induction by Ara-C, Adriamycin, methotrexate and vinblastine, as detected by cDNA-PCR (FIGS. 5A through 5C) and dye efflux assays with Ara-C treated cells. To investigate the specificity of the observed inhibition for PKC, effects of increasing doses of H7 (IC.sub.50 =6.0 .mu.M for PKC, 3.0 .mu.M for protein kinase A) and HA1004, a non-PKC specific protein kinase inhibitor (IC.sub.50 =40 .mu.M for PKC, 2.3 .mu.M for protein kinase A) (Hidaka et al., 1984, Biochemistry 23: 5036-5041) were compared. As shown in FIG. 5A, H7 inhibited MDR1 induction by Ara-C at 10 .mu.M or higher concentration, but HA1004 showed no significant inhibition even at 60 .mu.M. These results were consistent with a role for PKC in MDR1 induction by cytotoxic drugs.

Detailed Description Paragraph Right (69):
To further evaluate the capacity of protein kinase inhibitors to suppress MDR1 induction by cytotoxic drugs and PKC agonists, H-9 human T-cell leukemia cells were used in cDNA-PCR assays to examine induced MDR1 mRNA expression levels in the presence of protein kinase inhibitors. The results of these assays are shown in FIGS. 7A through 7F and are summarized in accompanying Table II. MDR1 was induced in cells represented in FIG. 7F by overnight incubation in 10 ng/mL TPA; 10 hour incubation with 25 .mu.M Ara-C was used to induce MDR1 expression in all other cell cultures analyzed as in FIG. 7A through 7E.

Detailed Description Paragraph Right (70):
Two erbstatin analogues (the tyrphostins A25 and B46) were tested for the ability to inhibit Ara-C induced MDR1 mRNA expression, as shown in FIG. 7A. Both of these compounds (tyrphostin A25, Lanes 5 and 6; tyrphostin B46, Lanes 7 and 8) demonstrated the capacity to strongly inhibit Ara-C induced MDR1 expression when incubated with H-9 cells at concentrations of 50-100 .mu.M.

Detailed Description Paragraph Right (71):
The concentration dependence of the ability of the erbstatin analog methyl-2,5-dihydroxycinnamate to inhibit MDR1 expression induced by treatment of H-9 cells with 25 .mu.M Ara-C is shown in FIG. 7C. These results demonstrate that this erbstatin analog inhibits MDR1 expression at both 32 .mu.M and 64 .mu.M to essentially the same degree.

Detailed Description Paragraph Right (72):
Inhibition of Ara-C induced MDR1 expression by the combination of calphostin C and direct illumination with white light in shown in FIG. 7D. This compound was found to be capable of inhibiting MDR1 expression when present at concentration of 1 .mu.M (Lanes 7 and 8); the inhibitory capacity of this compound was greatly reduced when the concentration used was reduced to 0.1 .mu.M (Lanes 5 and 6).

Detailed Description Paragraph Right (73):
Progressive dose-dependent inhibition of Ara-C induced MDR1 expression was demonstrated for the PKC inhibitor chelerythrine, shown in FIG. 7E. The dose-dependence of inhibition using this compound is seen by a comparison of the intensity of the MDR1-specific band in Lanes 5 and 6 (1 .mu.M chelerythrine) and Lanes 7 and 8 (5 .mu.M chelerythrine).

Detailed Description Paragraph Right (74):
FIG. 7F shows the results of incubation of H-9 cells in the presence of 30 nM staurosporine (Lanes 7 and 8) or 32 .mu.M methyl-2,5-dihydroxycinnamate (erbstatin analog) on the induction of MDR1 expression by TPA, a known PKC agonist. TPA alone (Lanes 3 and 4) efficiently induced readily detectable levels of MDR1 expression, consistent with the results disclosed above. Both staurosporine, a PKC inhibitor, and methyl-2,5-dihydroxycinnamate, completely inhibited TPA-induced MDR1 expression. These results are in agreement with previous observation that erbstatin analogues, which are known to inhibit epidermal growth factor tyrosine kinase, also have PKC inhibitory activity (Bishop et al., ibid.).

Detailed Description Paragraph Right (75):
A number of other protein kinase inhibitors were found to have little or no capacity to inhibit Ara-C induced MDR1 expression. These compounds include D,L-threo-sphingosine (tested at 5 .mu.M; higher concentrations induced cellular toxicity) and herbimycin A (tested at concentrations from 0.35-3 .mu.M). No significant inhibition was observed with Iso-H7, a structural analog of H7 with 10 fold weaker effect on protein kinases (Pelosin et al., 1990, Biochem. Biophys. Res. Commun. 169: 1040-1048). Similar results were observed with Ara-C treated K562 cells. Another protein kinase inhibitor, genistein (150 .mu.M) was found to only weakly inhibit Ara-C induced MDR1 expression (Table II).

Detailed Description Paragraph Right (76):
These results indicated that certain among a number of protein kinase inhibitors, and particularly protein kinase C inhibitors, were capable of inhibiting MDR1 expression induced by treatment of cancer cells with cytotoxic drugs or protein kinase C agonists. The data presented herein demonstrated that different chemotherapeutic drugs, including those that are not transported by P-glycoprotein, induced MDR1 expression directly, rather than by selection of preexisting genetic variants. Drug-induced MDR1 expression was limited to a subpopulation of treated cells and was associated with a moderate increase in the resistance of P-glycoprotein-transported drugs (approximately 2-3 fold in the case of K562 cells). This increase may be sufficient to reduce the response to chemotherapy in vivo and to enhance the selection of genetic mutants with higher levels of drug resistance. Drug-mediated induction of MDR1 expression may occur during cancer chemotherapy, and it may largely account for the increased incidence of MDR1 expression in treated tumors. Hence, the demonstration that PKC inhibitors can prevent MDR1 induction suggested using such agents in combination with cytotoxic drugs in cancer chemotherapy in order to achieve a higher degree of eradication of cancer cells.

Detailed Description Paragraph Right (77):
As demonstrated in Example 4, the antibacterial agent neomycin sulfate was tested for inhibitory capacity against Ara-C induced MDR1 expression. This compound was found to inhibit MDR1 expression in cells induced with 25 .mu.M Ara-C at concentrations of 10 mM (FIG. 7B, Lanes 7 and 8). These results demonstrate that neomycin sulfate possesses MDR1 inhibitory capacity at clinically relevant and achievable concentration. One of several known activities of neomycin sulfate is inhibition of PI-PLC (Cockroft et al., 1987, ibid.). These results suggested that other phospholipase C inhibitors could inhibit cytotoxic drug induction of MDR1 expression. Accordingly, cDNA-PCR assays were performed as described above in Example 4 on H9 human leukemia cells in which MDR1 gene expression was induced using AraC (25 .mu.M), in the presence or absence of phospholipase C inhibitors.

Detailed Description Paragraph Right (79):
After each incubation was complete, steady-state levels of MDR1- and .beta..sub.2 -microglobulin mRNA (internal standard) were determined by RT-PCR as described above in

Example 4. PCR products were then analyzed in 7.5% polyacrylamide gel electrophoresis and autoradiographed. The results of these experiments are shown in FIG. 8A. Lane 1 shows cDNA-PCR of MDR1 mRNA (the upper band, 167 bp) and .beta..sub.2 -microglobulin mRNA (the lower band, 120 bp) in H9 cells untreated with either AraC or U73122. In these cells, the band corresponding to .beta..sub.2 -microglobulin mRNA was detected, but no MDR1 mRNA-specific band was seen, consistent with the results on uninduced H9 cells shown above in Example 4 and FIGS. 7A through 7F. Lane 2 shows the results of cDNA-PCR analysis of H9 cells treated with 25 .mu.M AraC; in these cells a clear MDR1-specific band was detected. Lanes 3 and 4 show the results of cDNA-PCR on H9 cell mRNA treated in the presence of 1 and 10 .mu.M U73122, respectively. Autoradiographic analysis of the intensity of the MDR1-specific band revealed that U73122 inhibited the induction of MDR1 mRNA in these cells by AraC.

Detailed Description Paragraph Right (80):
The specificity of phospholipase C-targeted inhibition of MDR1 induction by cytotoxic drugs was determined in a comparative experiment using a known inhibitor of phosphocholine-dependent phospholipase C (PC-PLC). This compound, tricyclodecan-9-yl-xanthogenate potassium (D609) was found to be incapable of inhibiting AraC-induced expression of MDR1 even at concentrations (100 .mu.g/mL) known to be sufficient to inhibit PC-PLC.

Detailed Description Paragraph Right (81):
These results indicated that certain among a number of phospholipase C inhibitors, and specifically phosphoinositol-dependent PLC inhibitors, were capable of inhibiting MDR1 expression induced by treatment of cancer cells with cytotoxic drugs. This demonstration that PI-PLC inhibitors can prevent MDR1 induction suggested using such agents in combination with cytotoxic drugs in cancer chemotherapy in order to prevent development of MDR1-mediated drug resistance.

Detailed Description Paragraph Right (82):
In view of the results disclosed above, other intracellular components of eukaryotic signal transduction pathways were examined as targets of MDR1 gene induction by cytotoxic drugs. In this regard, agents which increased intracellular Ca.sup.2+ ion concentration were assayed for the ability to induce MDR1 gene expression.

Detailed Description Paragraph Right (84):
After each incubation was complete, steady-state levels of MDR1- and .beta..sub.2 -microglobulin mRNA (internal standard) were determined by RT-PCR as described above in Example 4. PCR products were then analyzed in 7.5% polyacrylamide gel electrophoresis and autoradiographed. The results of these experiments are shown in FIG. 8B. Lane 1 shows cDNA-PCR bands specific for MDR1 mRNA and .beta..sub.2 -microglobulin mRNA as above in Example 5 in untreated H9 cells. The absence of the MDR1-specific band and presence of the .beta..sub.2 -microglobulin specific band was consistent with the results on uninduced H9 cells shown above in Examples 4 and 5 and FIGS. 7A through 7F. Lane 2 shows the results of a similar cDNA-PCR analysis of H9 cells treated with 10 .mu.M A23187, and Lane 3 shows the cDNA-PCR results from H9 cells treated with 10 .mu.g/mL thapsigargin. In these cells a clear MDR1-specific band was detected, indicating that an increase in intracellular levels of calcium, an event associated with signal transduction mechanisms in eukaryotic cells, was capable of inducing MDR1 gene expression, which capacity had been discovered for treatment of H9 cells with both cytotoxic drugs and PKC agonists (see Examples 3 and 4, above).

Detailed Description Paragraph Right (85):
In view of these results, cytoplasmic calcium antagonists and calmodulin inhibitors were tested for the ability to inhibit induction of MDR1 gene expression by a variety of cytotoxic drugs. H9 cells were incubated as described above in the presence of the following cytotoxic drugs: Ara C (at a concentration of 25 .mu.M), Adriamycin (2 .mu.M), bleomycin (100 .mu.g/ml), 2-deoxyglucose (10 mM) and nocodazole (2.5 .mu.M). In parallel, cultures of H9 cells were incubated in the presence of each of these cytotoxic drugs and of a highly specific chelator of intracellular calcium ion, 1, 2-bis(o-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid, tetra(acetoxymethyl)-ester (BAPTA/AM) at a concentration of 5 .mu.M for 10 hrs. under the conditions described above. The inhibitor was added 45 min. prior to the addition of each of the MDR1 inducing agents. cDNA-PCR analysis, including gel electrophoresis and autoradiography as described above, was performed for each culture after drug treatment in the presence or absence of this chelator. The results of these experiments are shown in FIG. 8C.

Detailed Description Paragraph Right (86):
Each lane in FIG. 8C is labeled to indicate the identity of the cytotoxic drug and the

presence (+) or absence (-) of BAPTA/AM in the incubation media. These results demonstrate that pretreatment of H9 cells with 5 .mu.M BAPTA/AM completely abrogated the induction of MDR1 expression by different cytotoxic agents. This inhibitory effect of BAPTA/AM was detectable when the chelator was given to the cells within first 3 hrs. prior to the addition of MDR1 inducers.

Detailed Description Paragraph Right (87):
In another series of experiments, an inhibitor of intracellular release of calcium ions from cytoplasmic stores, 8-(dimethylamino)octyl-3,4,5-trimethoxybenzoate hydrochloride (TMB-8), was tested as described above and found to completely abrogate the ability of AraC to induce MDR1 gene expression in H9 cells when present at a concentration of 200 .mu.M.

Detailed Description Paragraph Right (88):
Having demonstrated that BAPTA/AM and TMB-8 could inhibit cytotoxic drug induction of MDR1 gene expression, an inhibitor of a major mediator of the calcium-dependent signal transduction mechanism, calmodulin, was assayed. H9 cells were incubated as above with 25 .mu.M AraC in the presence or absence of 10 .mu.M calmidazolium chloride, and cDNA-PCR analysis as described above was performed. The results of these experiments are shown in FIG. 8D. Calmidazolium chloride was found to efficiently abrogate AraC induction of MDR1 expression under the conditions of this assay. These results, taken with the results of BAPTA/AM inhibition disclosed above, indicate that inhibition of intracellular calcium ion release prevents induction of MDR1.

Detailed Description Paragraph Right (89):
This demonstration of the role of cytoplasmic calcium ion concentration and calcium-dependent signal transduction on induction of MDR1 gene expression suggested that such agents can be used in combination with cytotoxic drugs in cancer chemotherapy in order to prevent development of MDR1-mediated drug resistance.

Detailed Description Paragraph Right (90):
In view of the efficient induction of MDR1 gene expression by AraC, cellular responses to AraC treatment were investigated as targets, the inhibition of which could abrogate MDR1 induction by AraC. Recently it has been reported that treatment of cells with Ara C results in the release of NF-.kappa.B from the inhibitory subunit followed by translocation of the active NF-.kappa.B to the nucleus (see Siebenlist et al. and Kopp et al., ibid.) This suggested that agents which inhibit the activation of NF-.kappa.B (for example, as the result of stress stimuli) would also be able to prevent induction of MDR1 by chemotherapeutic drugs.

Detailed Description Paragraph Right (91):
Accordingly, MDR1 gene expression was induced as above in 9 cells with AraC for 10 hrs. in the presence or absence of 20 .mu.M pyrrolidone dithiocarbamate, 25 .mu.M N-tosyl-L-phenylalanine chloromethyl ketone, 20 .mu.M sodium salicylate or 10 mM acetylsalicylic acid (aspirin). All inhibitors were given 45 min. prior to Ara C treatment. cDNA-PCR analysis was performed as described above, and the results of these experiments shown in FIG. 8E.

Detailed Description Paragraph Right (92):
Each of the inhibitors tested completely abrogated the induction of MDR1 gene expression by 25 .mu.M AraC. Untreated H9 cells showed the expected pattern of .beta.2-microglobulin expression without detectable MDR1 expression (lane 1), and AraC treated cells showed expression of both genes (lane 2). H9 cells pretreated with 20 .mu.M pyrrolidone dithiocarbamate (lane 3), 25 .mu.M N-tosyl-L-phenylalanine chloromethyl ketone (lane 4), 20 mM sodium salicylate (lane 5) or 10 mM aspirin (lane 6) all showed complete suppression of AraC-induced MDR1 expression.

Detailed Description Paragraph Right (93):
This experiment was repeated using 2 .mu.M Adriamycin instead of Ara C to induce MDR1 expression As shown in FIG. 8F, essentially the same results obtained using AraC were obtained for each of the inhibitors tested when MDR1 expression was induced by Adriamycin.

Detailed Description Paragraph Right (94):
These results indicated that certain inhibitors of NF-.kappa.B activation, including aspirin, were capable of inhibiting MDR1 expression induced by treatment of cancer cells with cytotoxic drugs. These data suggested using such agents in combination with cytotoxic drugs in cancer chemotherapy in order to prevent development of MDR1-mediated drug resistance. Sodium salicylate and aspirin are already in clinical use, for the

attenuation of inflammatory symptoms in patient with infectious and autoimmune diseases and for a broad spectrum of more recently-appreciated therapeutical properties (see Marnett, 1992, Cancer Res. 52: 5575-5589; Anderson et al., 1993, Cancer Res. 53: 806-809; Rao et al., 1995, Cancer Res. 55: 1464-1472). The use of such compounds in combination with administration of cancer chemotherapeutic drugs provides the capacity to increase the clinical efficacy of current antineoplastic treatment protocols.

Detailed Description Paragraph Right (95):
1.1 MRP Expression Inhibition Assays

Detailed Description Paragraph Right (98):
H-9 cells were used to analyze the effect of various protein kinase inhibitors on the expression of the multidrug resistance associated protein MRP (Grant et al., ibid.). The results of these experiments are shown in FIGS. 9A. When MRP mRNA expression was evaluated in H-9 cells in the absence of any drugs, these cells were found to express the MRP gene robustly (FIGS. 9A, Lanes 1 and 2). In contrast to MDR1, incubation of H-9 cells in 25 .mu.M Ara-C resulted in a slight decrease in MRP expression (data not shown). Following incubation with staurosporine (100 nM; Lanes 3 and 4), chelerythrine (1-5 .mu.M; Lanes 5 and 6); methyl-2,5-dihydroxycinnamate (32 .mu.M; Lanes 7 and 8) or neomycin sulfate (10 mM; Lanes 9 and 10), MRP expression was strongly inhibited.

Detailed Description Paragraph Right (99):
These results demonstrate that a variety of protein kinase inhibitory compounds have the capacity to inhibit both cytotoxic drug-induced MDR1 expression and expression of the multidrug resistance phenotype-related MRP gene. These results strongly suggest that such protein kinase inhibitors have great utility in preventing the emergence of multidrug resistance in cancer patients receiving chemotherapeutic treatment.

Detailed Description Paragraph Left (1):
1. Induction of MDR1 Gene Expression

Detailed Description Paragraph Left (2):
2. Use of Protein Kinase Inhibitors to Prevent MDR1 Induction

Detailed Description Paragraph Left (3):
3. Use of Phosphoinositol-dependent Phospholipase C Inhibitors to Inhibit MDR1 Expression

Detailed Description Paragraph Left (4):
4. Use of Cytoplasmic Calcium Antagonists and Calmodulin Inhibitors to Inhibit MDR1 Expression

Detailed Description Paragraph Left (5):
5. Use of Inhibitors of Transcription Factor NF-.kappa.B Activation to Inhibit MDR1 Expression

Detailed Description Paragraph Left (6):
6. Use of Protein Kinase Inhibitors to Inhibit MRP Expression

Detailed Description Paragraph Left (8):
(where .beta..sub.2 M represents .beta..sub.2 microglobulin). PCR was performed for .beta..sub.2 M experiments under a regime consisting of 1 cycle comprising denaturation at 94.degree. C. for 3 minutes, primer annealing at 60.degree. C. for 30 seconds, and primer extension at 72.degree. C. for 1 minute, followed by 19 cycles comprising denaturation at 94.degree. C. for 30 seconds, primer annealing at 60.degree. C. for 30 seconds, and primer extension at 72.degree. C. for 1 minute, followed by a final cycle comprising denaturation at 94.degree. C. for 30 seconds, primer annealing at 60.degree. C. for 30 seconds, and primer extension at 72.degree. C. for 5 minutes. MDR1 cDNA sequences were amplified using the identical protocol as for .beta..sub.2 M, but extending the amplification from 19 cycles to 25 cycles. In addition, the cDNA-PCR amplification protocol contained the following modifications: (i) Taq DNA polymerase was added to the PCR mixtures after initial heating of the samples to 94.degree. C. (ii) The yield of the .beta..sub.2 -microglobulin-specific band, obtained after 22-28 cycles of PCR, was used as the primary criterion for equalizing the starting amounts of the cDNA templates in different preparations, in order to account for differential RNA degradation in cells subjected to different types of treatment. .sup.32 P-labeled PCR products were detected by autoradiography.

Detailed Description Paragraph Left (9):

However, staurosporine did not induce MDR1 expression in P-glycoprotein-negative H9 cells. The addition of staurosporine to H9 cells 30 minutes prior to TPA or DOG treatment completely abolished MDR1 induction by these agents, as evidenced by flow cytometric (FIGS. 1A through 1E) and cDNA-PCR assays (FIG. 2A). Staurosporine also inhibited the effects of TPA and DOG in normal PBL.

Detailed Description Paragraph Left (12):
(where .beta..sub.2 M represents .beta..sub.2 microglobulin). PCR was performed for .beta..sub.2 M experiments under a regime consisting of 1 cycle comprising denaturation at 94.degree. C. for 3 minutes, primer annealing at 60.degree. C. for 30 seconds, and primer extension at 72.degree. C. for 1 minute, followed by 19 cycles comprising denaturation at 94.degree. C. for 30 seconds, primer annealing at 60.degree. C. for 30 seconds, and primer extension at 72.degree. C. for 1 minute, followed by a final cycle comprising denaturation at 94.degree. C. for 30 seconds, primer annealing at 60.degree. C. for 30 seconds, and primer extension at 72.degree. C. for 5 minutes. MRP cDNA sequences were amplified using a protocol consisting of 1 cycle comprising denaturation at 94.degree. C. for 3 minutes, 26 cycles of denaturation at 94.degree. C. for 30 seconds, primer annealing at 62.degree. C. for 30 seconds, and primer extension at 72.degree. C. for 1 minute, followed by 1 cycle comprising primer extension at 72.degree. C. for 5 minutes, resulting in an MRP-specific amplified fragment of 292 bp. .sup.32 P-labeled PCR products were detected by autoradiography.

Detailed Description Paragraph Center (3):
A Protein Kinase Inhibitor Prevents Protein Kinase C Agonist-mediated MDR1 Induction in Normal and Tumor Cells

Detailed Description Paragraph Center (7):
Protein Kinase Inhibitors Prevent Cytotoxic Drug-mediated MDR1 Induction in Tumor Cells

Detailed Description Paragraph Center (17):
Protein Kinase Inhibitors Prevent MRP Expression in Tumor Cells

Detailed Description Paragraph Center (19):
Functional Assays of MRP Expression in Cells Treated with Protein Kinase Activators and Inhibitors

Detailed Description Paragraph Table (1):
.beta..sub.2 M (sense) 5'-ACCCCCACTGAAAAAGATGA-3' (SEQ ID No.: 1) .beta..sub.2 M (antisense) 5'-ATCTTCAAACCTCCATGATG-3' (SEQ ID No.: 2) MDR1 (sense) 5'-CCCATCATTGCAATAGCAGG-3' (SEQ ID No.: 3) MDR1 (antisense) 5'-GTTCAAACTTCTGCTCCTGA-3' (SEQ ID No.: 4)

Detailed Description Paragraph Table (2):
TABLE I EFFECT OF TPA ON MDR1 EXPRESSION Cell Line Untreated TPA-Treated Assays Normal Cells PBL + ++ F,A,R Human Hematopoietic Cell Lines KG1 (acute myelogenous leukemia) ++ +++ F,A KG1a (acute myelogenous leukemia) ++ +++ F,A K562 (chronic myelogenous - ++ F,A,R leukemia) H9 (T-cell leukemia) - ++ F,A,R HL-60 (promyelocytic leukemia) - - F THP-1 (promyelocytic leukemia) - - F Jurkat, clone E6-1 (T-cell leukemia) - - F Molt-4 (T-cell leukemia) - - F U937 (histiocytic leukemia) - - F Mouse Hematopoietic Cell Lines EM (thymoma) ++ +++ F LBRM 33, clone 4A2 (lymphoma) + ++ F Human Solid Tumor Cell Lines EJ (bladder carcinoma) + ++ F MCP-7 (breast carcinoma) - + R HeLa (cervical carcinoma) - - F,R KB-3-1 (HeLa subline) - - F,R HT 1080 (fibrosarcoma) - - F,R MDR1 gene expression was evaluated by a functional assay for Rh123 accumulation (F), UIC2 antibody staining (A) or cDNA-PCR assay for MDR1 mRNA (R), and expressed as relative values. Cells were considered negative if they expressed no P-glycoprotein detectable by the Rh123 or UIC2 staining assays and had MDR1 mRNA level no higher than that of KB-3-1 cells.

Detailed Description Paragraph Table (3):
TABLE II Inhibition of Ara-C Induced MDR1 Expression by Protein Kinase Inhibitors Compound Conc. tested Results herbimycin A (12 h) 0.3-3 .mu.M - tyrphostin A25 (16 hr) 50-100 .mu.M +++ tyrphostin B46 (16 hr) 50-100 .mu.M +++ D,L-threo-sphingosine 5 .mu.M - genistein 150 .mu.M + staurosporine 30 nM +++ H7 50 .mu.M +++ calphostin C (+ light) 1 .mu.M +++ chelerythrine 5 .mu.M +++ methyl-1,5-dihydroxycinnamate 32 .mu.M ++++ neomycin sulfate 10 mM +++ MDR1 induced by incubation of H-9 cells with 25 .mu.M Ara-C for 10 h: ++++ = complete inhibition of Ara-C mediated induction; + = weak inhibition of Ara-C mediated induction; - = no inhibition of Ara-C mediated induction

Detailed Description Paragraph Table (4):
.beta..sub.2 M (sense) 5'-ACCCCCACTGAAAAAGATGA-3' (SEQ ID No.: 1) .beta..sub.2 M (antisense) 5'-ATCTTCAAACCTCCATGATG-3' (SEQ ID No.: 2) MRP (sense) 5'-GGACCTGGACTTCGTTCTCA-3' (SEQ ID No.: 5) MRP (antisense) 5'-CGTCCAGACTTCCTTCATCCG-3' (SEQ ID No.: 6)

Detailed Description Paragraph Table (5):
TABLE III Inhibition of MRP Expression by Protein Kinase Inhibitors Compound Conc. tested Results staurosporine 100 nM ++++ chelerythrine 1-5 .mu.M ++++ methyl-1,5-dihydroxycinnamate 32 .mu.M ++++ neomycin sulfate 10 mM ++++ ++++ = complete inhibition of MRP expression

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Other Reference Publication (188):
Ueda et al., 1987, "The Human Multidrug Resistance (mdr1) Gene," J. Biol. Chem. 262:505-508.

CLAIMS:

1. A method of inhibiting MDR1 induction in a cancer cell by treatment with a cytotoxic drug, comprising contacting the cell with a phosphoinositol-dependent phospholipase C inhibitor coincident with treatment with the cytotoxic drug.

2. The method according to claim 1, wherein the phosphoinositol-dependent phospholipase C inhibitor is neomycin sulfate, 1-(6-((17.beta.-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl)-1-H-pyrrole-2,5-dione, or phospholipase C-inhibiting analogues thereof.

4. The method of claim 1 wherein the cancer cells contain little or no detectable MDR1-encoded P-glycoprotein, as determined by immunoreactivity with anti-P-glycoprotein antibodies, accumulation or efflux of P-glycoprotein transported dyes, or MDR1 mRNA expression assay.

7. A method of inhibiting MDR1 induction in a cancer cell by treatment with a cytotoxic drug, comprising contacting the cell with an cytoplasmic calcium antagonist or calmodulin inhibitor, coincident with treatment with the cytotoxic drug.

8. The method according to claim 7, wherein the cytoplasmic calcium antagonist is

1,2-bis(o-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid, tetra(acetoxymethyl)-ester, 8-(dimethylamino)octyl-3,4,5-trimethoxybenzoate hydrochloride, or analogues thereof that are cytoplasmic calcium ion antagonists.

9. The method according to claim 7, wherein the calmodulin inhibitor is 1-{6-{{17.beta.-3-methoxyestra-1,3,5(10)-trien-17-yl}amino}hexyl}-1-H-pyrrole-2,3-dione, or calmodulin-inhibiting analogues thereof.

11. The method of claim 7 wherein the cancer cells contain little or no detectable MDR1-encoded P-glycoprotein, as determined by immunoreactivity with anti-P-glycoprotein antibodies, accumulation or efflux of P-glycoprotein transported dyes, or MDR1 mRNA expression assay.

14. A method of inhibiting MDR1 induction in a cancer cell by treatment with a cytotoxic drug, comprising contacting the cell with an inhibitor of activation of eukaryotic transcription factor NF-.kappa.B, coincident with treatment with the cytotoxic drug.

15. The method according to claim 14, wherein the inhibitor of activation of eukaryotic transcription factor NF-.kappa.B is pyrrolidone dithiocarbamate, N-tosyl-L-phenylalanine chloromethyl ketone, sodium salicylate or acetysalicylic acid, or analogues thereof that inhibit activation of eukaryotic transcription factor NF-.kappa.B.

17. The method of claim 14 wherein the cancer cells contain little or no detectable MDR1-encoded P-glycoprotein, as determined by immunoreactivity with anti-P-glycoprotein antibodies, accumulation or efflux of P-glycoprotein transported dyes, or MDR1 mRNA expression assay.

20. A pharmaceutical composition comprising therapeutically-effective amount of a phosphoinositol-dependent phospholipase C inhibitor and a pharmaceutically-acceptable carrier.

21. A pharmaceutical composition comprising therapeutically-effective amount of an cytoplasmic calcium antagonist or calmodulin inhibitor, and a pharmaceutically-acceptable carrier.

22. A pharmaceutical composition comprising therapeutically-effective amount of an inhibitor of activation of eukaryotic transcription factor NF-.kappa.B, and a pharmaceutically-acceptable carrier.

WEST Search History

DATE: Thursday, May 02, 2002

Set Name side by side	Query	Hit Count	Set Name result set
	DB=JPAB,EPAB,DWPI; PLUR=NO; OP=ADJ		
L65	L64 and ((drug or multidrug) adj resistan$2)	2	L65
L64	semaphorin or (B adj 94) or (mel adj 14) or (mel-14) or 24p3 or proliferin or maspin	113	L64
	DB=USPT; PLUR=NO; OP=ADJ		
L63	L62 and @ad<19990131	7	L63
L62	L61 with ((drug or multidrug) adj resistan$2)	7	L62
L61	semaphorin or (B adj 94) or (mel adj 14) or (mel-14) or 24p3 or proliferin or maspin	790	L61
	DB=JPAB,EPAB,DWPI; PLUR=NO; OP=ADJ		
L60	L59 and @pd<19990131	11	L60
L59	L58 not us[pc]	28	L59
L58	L57 and ((drug or multidrug) adj resistan$2)	48	L58
L57	(screen$3 or assay$3) with ((test adj compounds$1) or agent$1 or molecule$1)	10653	L57
	DB=USPT; PLUR=NO; OP=ADJ		
L56	L55 not l50	17	L56
L55	L54 or l53	21	L55
L54	L52 and @prad<19990131	3	L54
L53	L52 and @ad<19990131	21	L53
L52	L51 with ((drug or multidrug) adj resistan$2)	22	L52
L51	(screen$3) with ((test adj compounds$1) or agent$1 or molecule$1)	14489	L51
L50	L49 or l48	34	L50
L49	L47 and @prad<19990131	12	L49
L48	L47 and @ad<19990131	34	L48
L47	L42 with (modulat$3 or decreas$3 or inhibit$3 or downregulat$3 or antagoniz$3)	35	L47
L46	L45 or l44	14	L46
L45	L43 and @prad<19990131	5	L45
L44	L43 and @ad<19990131	14	L44
L43	L42[clm]	16	L43
L42	L41 with ((drug or multidrug) adj resistan$2)	170	L42

L41	(method$1 or assay$1) with ((test adj compounds$1) or agent$1 or molecule$1)	145084	L41
L40	method$1 with ((test adj compounds$1) or agent$1 or molecule$1)	140235	L40
L39	L37 and @prad<19990131	0	L39
L38	L37 and @ad<19990131	4	L38
L37	l32 with (mRNA or polynucleotide$1 or cDNA)	5	L37
L36	L35 or l34	9	L36
L35	L33 and @prad<19990131	1	L35
L34	L33 and @ad<19990131	9	L34
L33	L32[clm]	10	L33
L32	(method$1 or assay$1) with (modulat$4 or decreas$3 or underexpress$3 or antagoni$3) with ((drug or multidrug) adj resist$4)	48	L32
L31	(method$ or assay$1) with (modulat$4 or decreas$3 or underexpress$3 or antagoni$3) with ((drug or multidrug) adj resist$4)	48	L31
L30	L29 not l27	2	L30
L29	L28 and @ad<19990131	8	L29
L28	L25 same (differential$2 adj express$3)	9	L28
L27	L26 and @ad<19990131	6	L27
L26	L25 with (differential$2 adj express$3)	6	L26
L25	(multidrug or drug) adj resistan$2	5604	L25
L24	multidrug adj resistan$2	1033	L24
L23	drug adj resistan$2	5132	L23
L22	L21 or l20	18	L22
L21	L19 and @prad<19990131	1	L21
L20	L19 and @ad<19990131	18	L20
L19	L18 and (modulat$3 or inhibit$3 or antagon$4)[clm]	22	L19
L18	L16 and l17	72	L18
L17	method with (mRNA or polynucleotide or cDNA)[clm]	2291	L17
L16	PDZK1 or cMOAT or Pgp or MDR$1 or MRP or (P adj gylcoprotein$1)	2714	L16
L15	cMOAT and ((drug adj resistan$2) or MDR)	4	L15
DB=JPAB,EPAB; PLUR=NO; OP=ADJ			
L14	cMOAT	1	L14
DB=USPT; PLUR=NO; OP=ADJ			
L13	melanoma with MDR	9	L13
L12	L11 same gene$1	12	L12
L11	melanoma same (drug adj resistan$2)	61	L11

L10	melanoma with (drug adj resistan$2)	22	L10
DB=DWPI; PLUR=NO; OP=ADJ			
L9	cMOAT	3	L9
L8	l6 not us[pc]	178	L8
L7	L6 and (drug adj resistance)	0	L7
L6	moat	454	L6
DB=PGPB; PLUR=NO; OP=ADJ			
L5	L4 and (drug adj resistance)	2	L5
L4	moat	62	L4
DB=USPT; PLUR=NO; OP=ADJ			
L3	moat and (drug resistance)	1	L3
L2	L1 and (antibiotic$1 or antimycotic$1 or drug or antimicrobial$1)	1	L2
L1	5585277[pn]	1	L1

END OF SEARCH HISTORY

WEST

Generate Collection Print

L38: Entry 1 of 4 File: USPT May 9, 2000

DOCUMENT-IDENTIFIER: US 6060244 A
TITLE: Genes and genetic elements associated with sensitivity to chemotherapeutic drugs

DATE FILED (1):
19970909

Detailed Description Paragraph Right (40):
To test whether decreased khcs gene expression is associated with any naturally occurring mechanisms of drug resistance, an assay was developed for measuring khcs mRNA levels by cDNA-PCR. This assay is a modification of the quantitative assay described by Noonan et al., Proc. Natl. Acad. Sci. USA 87:7160-7164 (1990) for determining mdr-1 gene expression. The oligonucleotide primers had the sequences AGTGGCTGGAAAACGAGCTA [SEQ. ID. No. 19] and CTTGATCCCTTCTGGTTGAT [SEQ. ID. No. 20]. These primers were used to amplify a 327 bp segment of mouse khcs cDNA, corresponding to the anti-khcs GSE. These primers efficiently amplified the mouse cDNA template but not the genomic DNA, indicating that they spanned at least one intron in the genomic DNA. Using these primers, we determined that khcs mRNA is expressed at a higher level in the mouse muscle tissue than in the kidney, liver or spleen.

WEST

Generate Collection Print

L50: Entry 5 of 34 File: USPT Aug 29, 2000

DOCUMENT-IDENTIFIER: US 6111092 A
TITLE: Nucleic acid molecules encoding DRT111 and uses thereof

DATE FILED (1):
19980603

Detailed Description Paragraph Right (168):
Agents, or modulators which have a stimulatory or inhibitory effect on DRT111 activity (e.g., DRT111 gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (e.g., drug-resistance) associated with aberrant DRT111 activity. In conjunction with such treatment, the pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (e.g., drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of DRT111 protein, expression of DRT111 nucleic acid, or mutation content of DRT111 genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Detailed Description Paragraph Right (176):
In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant DRT111 expression or activity (e.g., the development of drug resistance), by administering to the subject an agent which modulates DRT111 expression or at least one DRT111 activity. Subjects at risk for a condition which is caused or contributed to by aberrant DRT111 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the DRT111 aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. For example, administration of a prophylatic agent to a cancer patient may prevent or delay the development of drug resistance in the patient's cancer cells. Depending on the type of DRT111 aberrancy, for example, a DRT111 agonist or DRT111 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

WEST

Generate Collection Print

L50: Entry 6 of 34 File: USPT Jul 18, 2000

DOCUMENT-IDENTIFIER: US 6090565 A
TITLE: Sphingoglycolipids as markers for multidrug resistant cancers

DATE FILED (1):
19971105

Detailed Description Paragraph Right (87):
In these embodiments, the present invention is directed to a method for determining the ability of a candidate substance to inhibit the synthesis of sphingoglycolipid content of multidrug resistant cancer cells and to concomitantly sensitize the cells to other chemotherapeutic agents, the method including generally the steps of (a) obtaining an MDR cancer cell; (b) admixing a candidate substance with the MDR cell; and (c) determining the ability of the candidate substance to inhibit the sphingoglycolipid content of the MDR cancer cell.

WEST

Generate Collection Print

L50: Entry 14 of 34 File: USPT Mar 2, 1999

DOCUMENT-IDENTIFIER: US 5877030 A
TITLE: Process for creating molecular diversity and novel protease inhibitors produced thereby

DATE FILED (1):
19950531

Detailed Description Paragraph Right (11):
Examples of core molecules also include biochemical compounds (e.g., adenine, thymine, guanine, cytidine, uracil, inosine, as well as analogs, nucleosides, and nucleotides of the foregoing nucleobases). In a particularly preferred embodiment, the core molecules are pharmaceutical molecules having a known biological activity. Optionally, the pharmaceutical core molecules are derivatized (e.g., by converting a reactive center of the pharmaceutical core molecule into a more potent nucleophile or electrophile (discussed below) to enhance reaction of the core molecule with a tool molecule containing a complementary functional group. For example, a pharmaceutical core molecule having an antibiotic activity can be reacted with a plurality of tool molecules to form a library of derivatized antibiotic molecules. The derivatized pharmaceutical molecule library can be screened using well-known colony inhibition assays to identify lead compounds which, for example, exhibit enhanced antibiotic potency and/or which confer multidrug resistance. Conventional and/or novel separation techniques and analytical methods (e.g., HPLC and mass spectroscopy) can be used to elucidate the structure of the novel lead molecules. (See, e.g., the Examples).

L50: Entry 17 of 34 File: USPT Jun 17, 1997

DOCUMENT-IDENTIFIER: US 5639595 A
TITLE: Identification of novel drugs and reagents

DATE FILED (1):
19931202

Detailed Description Paragraph Right (26):
In accordance with other preferred embodiments of the present invention, selection for inhibition of expression of a cell surface protein in mammalian cells is performed. Oligonucleotides which inhibit the in vivo expression of a cell surface protein, such as, for example, intercellular adhesion molecules, multidrug resistance proteins such as MDR and MRP, and oncogene products expressed on the cell surface, can be identified using this method. The expression vector containing the random oligonucleotide insert is transfected into a mammalian, preferably human, cell line, using calcium phosphate, electroporation or other standard methods. Where an oncogene product is the cell surface protein to be inhibited, the cell line is preferably a transformed cell line. Oligonucleotides are expressed by inducing the RNA polymerase which directs the transcription of the random insert. Individual cells expressing sequences which reduce the expression of the cell surface protein can be isolated from the remaining population using a selective antibody killing assay. This assay takes advantage of the antibody-mediated complement lysis reaction. Cells that express the protein on the cell surface will be eliminated by lysis following addition of antibody to the protein, and complement. Hood, L. E., Weissman, I. L., and W. B. Wood. Immunology pp. 161-164; Benjamin/Cummings Publishing Co., Inc., 1978. This allows the recovery of those cells in which expression of the cell surface protein has been inhibited by oligonucleotide. These cells are then recovered and amplified in culture and the oligonucleotides contained within them can be identified by plasmid isolation or PCR amplification and DNA sequencing of clonal populations of individual recovered cells.

L50: Entry 18 of 34 File: USPT Nov 5, 1996

DOCUMENT-IDENTIFIER: US 5571687 A
TITLE: Modulators of multidrug resistance transporters

DATE FILED (1):
19940607

Detailed Description Paragraph Right (3):
The ATPase assay can also be used to identify test compounds that inhibit P-gp activity, ie P-gp antagonists, which are also potential agents for reversing P-gp-mediated drug resistance. In this type of assay, the ability of a selected test compound to reduce P-gp-ATPase which has been stimulated by a drug known to elicit this activity (eg verapimil or farnesylcysteine methyl ester (FCME) is examined. Increasing concentrations of the selected test compound and a fixed amount of the stimulatory drug are mixed, added to the P-gp preparation, and ATPase activity determined. A dose-dependent inhibition of ATPase activity by the selected compound indicates an antagonist.

WEST

Generate Collection Print

L56: Entry 2 of 17 File: USPT Oct 2, 2001

DOCUMENT-IDENTIFIER: US 6297216 B1
TITLE: Compounds for reversing drug resistance

DATE FILED (1):
19970219

Detailed Description Paragraph Right (54):
Additional in vitro methods of screening the ability of a Reversin compound to act as a chemosensitizing agent for the reversal of multidrug resistance are provided by Ford et al. supra, at Tables 1-6.

Other Reference Publication (21):
Yoshimura et al., "Novel screening method for agents that overcome classical multidrug resistance in a human cell line," Cancer Letters, 50:45-51, 1990.

WEST

Generate Collection Print

L56: Entry 4 of 17 File: USPT Jan 30, 2001

DOCUMENT-IDENTIFIER: US 6180343 B1
TITLE: Green fluorescent protein fusions with random peptides

DATE FILED (1):
19981008

Detailed Description Paragraph Right (156):
Multiple drug resistance, and hence tumor cell selection, outgrowth, and relapse, leads to morbidity and mortality in cancer patients. Candidate libraries can be introduced into tumor cell lines (primary and cultured) that have demonstrated specific or multiple drug resistance. Bioactive peptides can then be identified which confer drug sensitivity when the cells are exposed to the drug of interest, or to drugs used in combination chemotherapy. The readout can be the onset of apoptosis in these cells, membrane permeability changes, the release of intracellular ions and fluorescent markers. The cells in which multidrug resistance involves membrane transporters can be preloaded with fluorescent transporter substrates, and selection carried out for peptides which block the normal efflux of fluorescent drug from these cells. Candidate libraries are particularly suited to screening for peptides which reverse poorly characterized or recently discovered intracellular mechanisms of resistance or mechanisms for which few or no chemosensitizers currently exist, such as mechanisms involving LRP (lung resistance protein). This protein has been implicated in multidrug resistance in ovarian carcinoma, metastatic malignant melanoma, and acute myeloid leukemia. Particularly interesting examples include screening for agents which reverse more than one important resistance mechanism in a single cell, which occurs in a subset of the most drug resistant cells, which are also important targets. Applications would include screening for peptide inhibitors of both MRP (multidrug resistance related protein) and LRP for treatment of resistant cells in metastatic melanoma, for inhibitors of both p-glycoprotein and LRP in acute myeloid leukemia, and for inhibition (by any mechanism) of all three proteins for treating pan-resistant cells.

WEST

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L56: Entry 5 of 17 File: USPT Nov 28, 2000

DOCUMENT-IDENTIFIER: US 6153380 A
TITLE: Methods for screening for transdominant intracellular effector peptides and RNA molecules

DATE FILED (1):
19970123

Detailed Description Paragraph Right (134):
Multiple drug resistance, and hence tumor cell selection, outgrowth, and relapse, leads to morbidity and mortality in cancer patients. Candidate libraries can be introduced into tumor cell lines (primary and cultured) that have demonstrated specific or multiple drug resistance. Bioactive agents can then be identified which confer drug sensitivity when the cells are exposed to the drug of interest, or to drugs used in combination chemotherapy. The readout can be the onset of apoptosis in these cells, membrane permeability changes, the release of intracellular ions and fluorescent markers. The cells in which multidrug resistance involves membrane transporters can be preloaded with fluorescent transporter substrates, and selection carried out for peptides which block the normal efflux of fluorescent drug from these cells. Candidate libraries are particularly suited to screening for peptides which reverse poorly characterized or recently discovered intracellular mechanisms of resistance or mechanisms for which few or no chemosensitizers currently exist, such as mechanisms involving LRP (lung resistance protein). This protein has been implicated in multidrug resistance in ovarian carcinoma, metastatic malignant melanoma, and acute myeloid leukemia. Particularly interesting examples include screening for agents which reverse more than one important resistance mechanism in a single cell, which occurs in a subset of the most drug resistant cells, which are also important targets. Applications would include screening for peptide inhibitors of both MRP (multidrug resistance related protein) and LRP for treatment of resistant cells in metastatic melanoma, for inhibitors of both p-glycoprotein and LRP in acute myeloid leukemia, and for inhibition (by any mechanism) of all three proteins for treating pan-resistant cells.

L56: Entry 6 of 17 File: USPT Jul 27, 1999

DOCUMENT-IDENTIFIER: US 5928637 A
TITLE: Methods of inducing multidrug resistance using human MDR1 cDNA

DATE FILED (1):
19950404

Detailed Description Paragraph Right (12):
The vectors of the present invention may be used in a wide variety of contexts. For example, one goal of chemotherapy research is to find new drugs that either overcome multidrug resistance making current agents more effective or that are not themselves substrates for the efflux pump. To search for such agents it is important to have cell lines that are identical except for their level of P-glycoprotein. The MDR1 vectors of the present invention now make it possible to transduce any drug sensitive cell line of interest, thereby rendering it drug resistant. Thus, for example, the MDR1 vectors of the present invention have been employed to transform colon (HT29), ovarian (OVCAR3) and melanoma (FEMX) cell lines. The resulting drug-resistant cell lines make excellent model systems for the screening therapeutic agents. Thus, in one embodiment, this invention provides for mammalian cells, preferably human cells transfected with a full-length human MDR1 cDNA that expresses a functional human p-glycoprotein and confers a multidrug resistant phenotype.

WEST

Generate Collection Print

L56: Entry 7 of 17 File: USPT Mar 2, 1999

DOCUMENT-IDENTIFIER: US 5876946 A
TITLE: High-throughput assay

DATE FILED (1):
19970603

Other Reference Publication (3):
Quesada et al., Chemosensitization and drug accumulation assays as complementary methods for the screening of multidrug resistance reversal agents, Cancer Letters 99 (1996) 109-114.

WEST

Generate Collection Print

L56: Entry 9 of 17 File: USPT Dec 22, 1998

DOCUMENT-IDENTIFIER: US 5851819 A
TITLE: Vectors carrying MDR1 cDNA which confer multidrug resistance on transduced cells

DATE FILED (1):
19950531

Detailed Description Paragraph Right (12):
The vectors of the present invention may be used in a wide variety of contexts. For example, one goal of chemotherapy research is to find new drugs that either overcome multidrug resistance making current agents more effective or that are not themselves substrates for the efflux pump. To search for such agents it is important to have cell lines that are identical except for their level of P-glycoprotein. The MDR1 vectors of the present invention now make it possible to transduce any drug sensitive cell line of interest, thereby rendering it drug resistant. Thus, for example, the MDR1 vectors of the present invention have been employed to transform colon (HT29), ovarian (OVCAR3) and melanoma (FEMX) cell lines. The resulting drug-resistant cell lines make excellent model systems for the screening therapeutic agents. Thus, in one embodiment, this invention provides for mammalian cells, preferably human cells transfected with a full-length human MDRI cDNA that expresses a functional human P-glycoprotein and confers a multidrug resistant phenotype.

WEST

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L60: Entry 2 of 11 File: EPAB Mar 2, 1995

DOCUMENT-IDENTIFIER: WO 9506132 A2
TITLE: IDENTIFYING BIOLOGICALLY ACTIVE AGENTS THROUGH CULTURE COLOR CHANGE

Publication Date (1):
19950302

Abstract (1):
The present invention relates to a method of identifying a biologically active agent, which affects a targeted cellular component, whose existence or function is essential for cell viability, pathogenesis or drug resistance. This assay method includes the use of a mixed culture of first and second cell strains. The cell strains may be either strains of different cells or different strains of the same cell type. The two cell strains are distinguished by color differences. The first cell strain has a first color while the second cell strain displays a second color. The second cell strain is also more sensitive towards a class of biologically active agents than is the first cell strain. The first and second cell strains, and a candidate agent are mixed with a medium, appropriate for growth of the strains, forming a mixed assay culture. The culture is then exposed to conditions appropriate for growth. The initial proportions of the first and second cell strains are such that the culture predominantly favors the development of the second color. The biological activity of the agent is then determined from the color of the culture. Where the mixed culture retains the second color, the agent was not active against the target. However, where the culture predominantly becomes the first color, then the agent showed specific activity towards the target.

WEST

Generate Collection Print

L60: Entry 6 of 11 File: DWPI Mar 11, 1998

DERWENT-ACC-NO: 1998-133341
DERWENT-WEEK: 199813

TITLE: New three-dimensional electric field pharmacophores - useful for screening and designing molecules with multi-drug resistance modulatory activity

Publication Date:
19980311

Basic Abstract Text:
Pharmacophore comprises a 3-dimensional array of field points defining a shape and volume. The set of these points is the aggregate average of the field points derived from several molecules which possess multidrug resistance (MDR) modulatory activity.

Basic Abstract Text:
USE - The pharmacophores are useful for screening and designing molecules having MDR modulatory activity (claimed) which are useful for treating multidrug resistant tumours, malaria, tuberculosis, leishmaniasis, amoebic dysentery and AIDS..

Standard Title Terms:
NEW THREE DIMENSION ELECTRIC FIELD USEFUL SCREEN DESIGN MOLECULAR MULTI DRUG RESISTANCE ACTIVE

Title (1):
New three-dimensional electric field pharmacophores - useful for screening and designing molecules with multi-drug resistance modulatory activity

Publication Date (1):
19980311

Basic Abstract Text (1):
Pharmacophore comprises a 3-dimensional array of field points defining a shape and volume. The set of these points is the aggregate average of the field points derived from several molecules which possess multidrug resistance (MDR) modulatory activity.

Basic Abstract Text (3):
USE - The pharmacophores are useful for screening and designing molecules having MDR modulatory activity (claimed) which are useful for treating multidrug resistant tumours, malaria, tuberculosis, leishmaniasis, amoebic dysentery and AIDS.

Standard Title Terms (1):
NEW THREE DIMENSION ELECTRIC FIELD USEFUL SCREEN DESIGN MOLECULAR MULTI DRUG RESISTANCE ACTIVE

WEST

Generate Collection Print

L60: Entry 8 of 11 File: DWPI Apr 27, 1995

DERWENT-ACC-NO: 1995-106860
DERWENT-WEEK: 199615

TITLE: Screening cpds. for biological activity on mixed cell culture - contg. cells of differing colours and sensitivities, partic. for detecting antifungal and antibiotic agents

Publication Date:
19950427

Publication Date:
19950302

Basic Abstract Text:
USE - The method is esp. used to evaluate antifungals or antibiotics, but also anticancer agents, immunosuppressants, RNA polymerase (inhibitors), insecticides and herbicides, etc., i.e. any agent targeted to a cell component essential for cell viability, pathogenicity or drug resistance.

Standard Title Terms:
SCREEN COMPOUND BIOLOGICAL ACTIVE MIX CELL CULTURE CONTAIN CELL DIFFER COLOUR SENSITIVE DETECT ANTIFUNGAL ANTIBIOTIC AGENT< br>
Title (1):
Screening cpds. for biological activity on mixed cell culture - contg. cells of differing colours and sensitivities, partic. for detecting antifungal and antibiotic agents

Publication Date (1):
19950427

Publication Date (2):
19950302

Basic Abstract Text (2):
USE - The method is esp. used to evaluate antifungals or antibiotics, but also anticancer agents, immunosuppressants, RNA polymerase (inhibitors), insecticides and herbicides, etc., i.e. any agent targeted to a cell component essential for cell viability, pathogenicity or drug resistance.

Standard Title Terms (1):
SCREEN COMPOUND BIOLOGICAL ACTIVE MIX CELL CULTURE CONTAIN CELL DIFFER COLOUR SENSITIVE DETECT ANTIFUNGAL ANTIBIOTIC AGENT

WEST

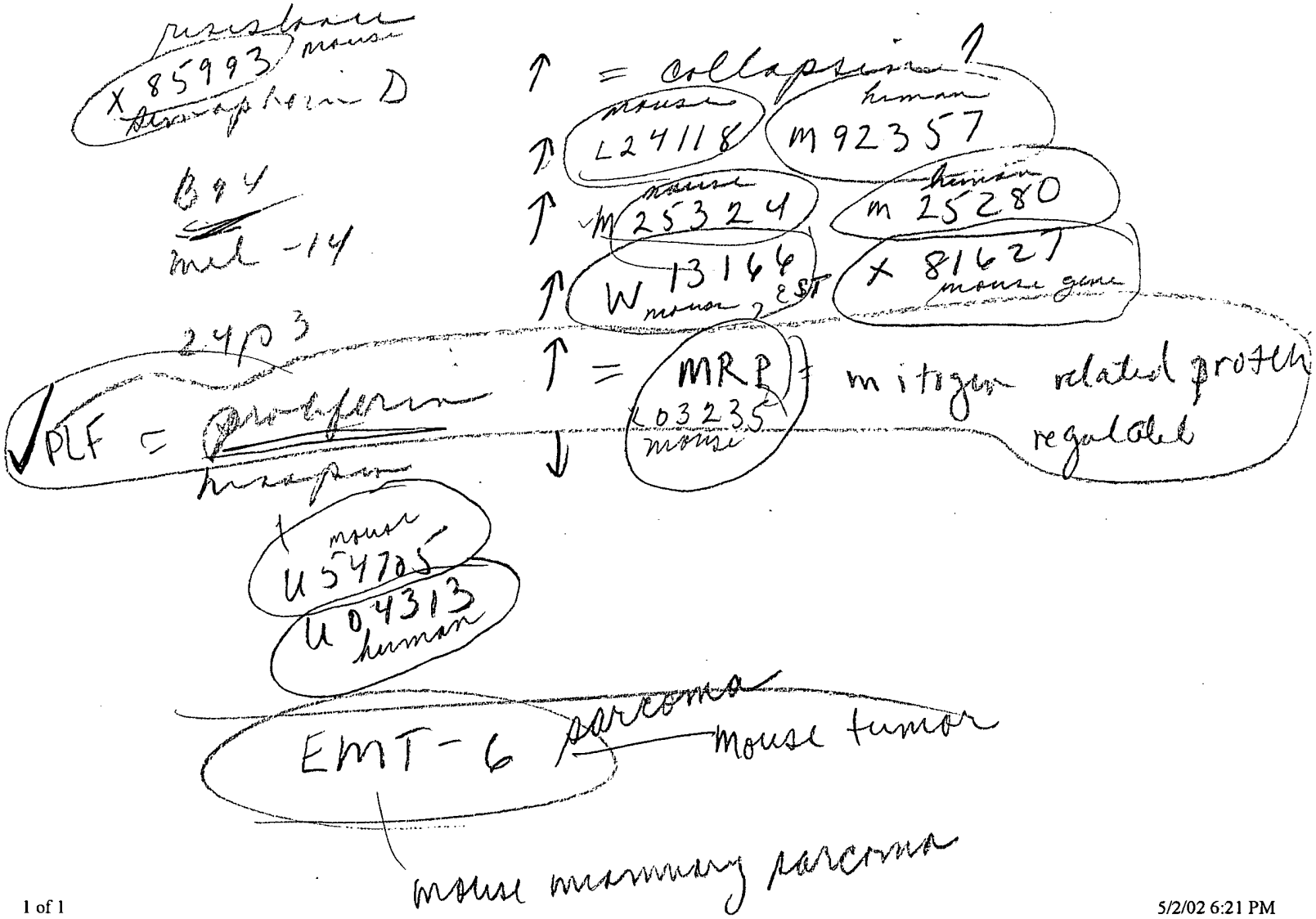
Generate Collection Print

L10: Entry 2 of 22 File: USPT May 22, 2001

DOCUMENT-IDENTIFIER: US 6235785 B1
TITLE: Methods for identifying and treating resistant tumors

Other Reference Publication (18):
"Membrane Transport Proteins Associated with Drug Resistance Expressed in Human Melanoma", Schadendorf, Makki, Stahr, van Dyck, Wanner, Scheffer, Flens, Scheper and Henz, American Journal of Pathology, vol. 147(6), Dec., 1995, pp. 1545-1552.

differentially expressed



WEST

Generate Collection Print

L9: Entry 1 of 3 File: DWPI Jul 24, 2000

DERWENT-ACC-NO: 2000-452526
DERWENT-WEEK: 200052

TITLE: Reducing chemotherapeutic drug resistance for treating an epithelial solid mass neoplasm comprises administering an antibody against an epitope of PDZK1 or canalicular multispecific organic anion transporter protein

INVENTOR: KOCHER, O N

PRIORITY-DATA: 1998US-0224623 (December 31, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 200025940 A	July 24, 2000		000	A61K000/00
WO 200040201 A2	July 13, 2000	E	056	A61K000/00

INT-CL (IPC): A61 K 0/00

ABSTRACTED-PUB-NO: WO 200040201A
BASIC-ABSTRACT:

NOVELTY - Reducing chemotherapeutic drug resistance exhibited in situ by a solid mass neoplasm of epithelial origin, comprises administering an antagonistic antibody against an epitope of PDZK1 protein or canalicular multispecific organic anion transporter (cMOAT) protein to inhibit in situ intracellular binding.

DETAILED DESCRIPTION - Reducing chemotherapeutic drug resistance comprises:

(1) identifying a solid mass neoplastic cell of epithelial origin as being constituted in part of tumor cells clinically resistant in situ to a previously administered chemotherapeutic treatment agent; and

(2) administering to the neoplasm an antagonistic antibody preparation specific against an epitope of PDZK1 protein or cMOAT protein so that intracellular binding of the proteins is inhibited in situ.

An INDEPENDENT CLAIM is also included for a composition for reducing chemotherapeutic drug resistance exhibited in situ by tumor cells constituting a solid mass neoplasm of epithelial origin, comprising an antagonistic antibody preparation specific against an epitope of PDZK protein or cMOAT so that intracellular binding of the proteins is inhibited in situ.

ACTIVITY - Anti-drug resistance; cytostatic. No biological data is given.

MECHANISM OF ACTION - PDZK1 inhibitor; cMOAT inhibitor. No biological data is given.

USE - The new method is used to reduce chemotherapeutic drug resistance exhibited in situ by a solid mass neoplasm of epithelial origin (claimed). The new method can be used on epithelial cell tumor of any kind, type, grade, age or stage, in vivo, such as breast, endometrial, colon, lung, kidney, and prostate cancers and squamous cell carcinoma of the skin.

ADVANTAGE - The effectiveness of chemotherapeutic drug treatments used clinically to treat humans and animals with solid mass cancers is enhanced.

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L15: Entry 3 of 4 File: USPT Jun 19, 2001

DOCUMENT-IDENTIFIER: US 6248752 B1
TITLE: Azabicyclooctane compositions and methods for enhancing chemotherapy

Abstract Paragraph Left (1):
The present invention relates to compositions and methods for enhancing the efficacy of therapeutic or prophylactic drugs. These compositions and methods are particularly useful to inhibit the abilities of drug transport proteins to efflux therapeutic agents from cells. Utilities of said compositions and methods include sensitizing drug resistant cells to anti-cancer agents, preventing the development of such drug resistance, enhancing the availability of therapeutic agents to the brain, testes, eyes and leukocytes, enhancing the oral bioavailability of therapeutic agents, sensitizing drug resistant infectious organisms to anti-infection agents, and preventing the development of such drug resistance.

Brief Summary Paragraph Right (3):
Two transport proteins which are important in the treatment of human diseases are termed P-glycoprotein (Pgp) and multidrug resistance-associated protein (MRP). Each of these proteins is highly effective in removing a variety of compounds from eucaryotic cells, using energy released by the hydrolysis of ATP. Because of their involvement in several human diseases, further discussed below, there is great interest in developing pharmaceutical agents which will effectively inhibit the abilities of these proteins to transport drugs. Additional transport proteins have been identified more recently, including cMOAT and additional proteins related to MRP.

Brief Summary Paragraph Right (6):
An example of transport protein-mediated drug resistance is the phenomenon of multidrug resistance (MDR) often encountered in cancer chemotherapy (Gottesman et al., Ann. Rev. Biochem. 62: 385 (1993)). In this situation, the proliferation of tumor cells that are resistant to many structurally unrelated drugs often results in the failure of chemotherapy. Tumor cells from patients undergoing chemotherapy often demonstrate elevated Pgp expression, suggesting that this mechanism of MDR is clinically important (Goldstein et al., J. Natl. Cancer Inst. USA 81: 116 (1989)). Recent studies have indicated that MRP is expressed in a high percentage of solid tumors and leukemias. However, no differences in MRP levels were detected between normal and malignant hematopoietic cells (Abbaszadegan et al., Cancer Res. 54: 4676 (1994)), and MRP levels were found to be lower in some tumors than in corresponding normal tissue (Thomas et al., Eur. J. Cancer 30A: 1705 (1994)). Therefore, it seems that different tumors will display different patterns of expression of Pgp, MRP and, possibly, other transporters.

Brief Summary Paragraph Right (10):
Organisms other than mammals also possess transport proteins similar to Pgp which have been shown to confer resistance to chemotherapeutic agents (Ullman, J. Bioenergetics Biomembranes 27: 77 (1995)). While the pharmacology of these transporters is not identical to that of Pgp, certain modulators are able to inhibit drug transport by both Pgp and protozoan transporters (Frappier et al., Antimicrob. Agents Chemother. 40: 1476 (1996)). It is envisioned that certain MDR modulators will facilitate drug accumulation in non-mammalian cells and so enhance the effectiveness of anti-infection chemotherapy.

Brief Summary Paragraph Right (11):
The preceding discussion demonstrates that drug transporters are involved in determining the success of chemotherapy in a variety of disease states. While a variety of compounds have been shown to reverse transporter-mediated MDR in cell culture (i.e. act as MDR modulators), the clinical success with these agents has been unimpressive,

predominantly due to the intrinsic toxicity of heretofore used modulators, and their undesired effects on the pharmacokinetics of the accompanying drugs. However, a likely cause of the failure of these agents is their lack of selectivity for different drug transporters. For example, inhibition of MRP by MDR modulators is likely to increase the uptake of cytotoxic anticancer drugs by many normal tissues, thereby producing greater untoward toxicity for the patient. Successful chemotherapy will consequently require a panel of transporter antagonists with differential selectivity for Pgp and MRP which will allow selection of the appropriate sensitizing agent.

Brief Summary Paragraph Right (12):
In accordance with one aspect, the present invention provides compounds and compositions which have been discovered to increase drug efficacy, and methods of chemosensitizing drug resistant cells using such compounds and compositions.

Brief Summary Paragraph Right (17):
These compounds, compositions and their use to inhibit drug transport from target cells and/or tissues are envisioned to have utility for treating MDR, including reversing MDR, chemosensitizing multidrug resistant cells to anti-cancer agents, as well as preventing MDR, by administering an effective amount of at least one of the compounds described herein to a patient in need of such treatment.

Brief Summary Paragraph Right (22):
The compounds included in the foregoing summary have been found to potentiate the cytotoxicity of anticancer drugs toward drug-resistant human cancer cells. Particularly preferred are compounds of the formula: ##STR3##

Brief Summary Paragraph Right (38):
The compounds of the invention can be used in various protocols for treating patients. For example, these compounds can be used in a method for treating tumor cells in a patient requiring such treatment. This method would involve administering to a cancer patient a compound as described above in an amount effective to attenuate drug resistance in such cells.

Brief Summary Paragraph Right (41):
Additionally, a compound of this invention may be administered in combination with an additional compound effective to sensitize drug resistant tumor cells, the amount of the combination being effective to enhance the therapeutic efficacy of the anticancer drug. The additional compound may be selected from the group consisting of dihydropyridines, thioxanthenes, phenothiazines, cyclosporins, acridonecarboxamides, verapamil, cyclosporin A, PSC 833, tamoxifen, quinidine, quinine, bepridil, ketoconazole, megestrol acetate and estramustine. Furthermore, new agents which inhibit drug efflux are described in the literature from time to time, and these compounds are also envisioned to provide useful combinations with those of the present invention.

Brief Summary Paragraph Right (42):
In view of the beneficial effect of reversal of MDR produced by the compounds of the invention, it is anticipated that these compounds will be useful not only for therapeutic treatment after the onset of MDR, but also for MDR prevention in patients about to undergo chemotherapy for the first time. The above-noted dosages will be essentially the same whether for treatment or prevention of MDR.

Detailed Description Paragraph Right (1):
The following example sets forth the test protocols for evaluating the MDR reversing activity of the modulating compounds described above, along with the test results. This example is provided for illustrative purposes only, and is not intended to limit the invention.

Detailed Description Paragraph Right (2):
The following cell lines were used in these studies: 1) MCF-7 human breast carcinoma cells; 2) MCF-7/ADR cells, an MDR subline which overexpresses Pgp (Fairchild et al. Cancer Res. 47: 5141 (1987) but not MRP; and 3) Human promyelocytic leukemia HL-60/ADR cells, which express MRP (Marsh et al. Cancer Res. 47: 4053 (1986)) but not Pgp.

Detailed Description Paragraph Right (3):
To test for reversal of Pgp-mediated MDR, MCF-7/ADR cells were placed into 96-well tissue culture plates at approximately 15% confluency, and were allowed to attach and recover for 24 hr. The cells were then treated with the varying concentrations of the described compounds in the presence of 0 or 25 nM actinomycin D, or 1 .mu.M daunomycin for 48 h according to previously described procedures (Smith et al. Oncology Res. 6:

211 (1994); Smith et al. Molec. Pharm. 47: 241 (1995)). After 48 h, cell survival was assayed using the sulforhodamine B binding assay (Skehan et al. J. Natl. Cancer Inst. USA 82: 1107 (1990)). The percentage of cells killed was calculated as the percentage decrease in sulforhodamine B binding compared with control cultures. Control cultures include equivalent amounts of ethanol (as the solvent control), which did not modulate the growth or drug-sensitivity of these cells at doses used in these studies. Inhibition of Pgp was manifested as the ability of the compound to potentiate the cytotoxicity of actinomycin D, daunomycin and/or vincristine toward MCF-7/ADR cells. To assess the toxicity of the compounds toward drug-sensitive cells, the effects of the test modulators on the growth of drug-sensitive MCF-7 cells were determined by the same methods.

Detailed Description Paragraph Right (4):
To test for reversal of MRP-mediated MDR, HL-60/ADR cells were treated with varying concentrations of the described compounds in the presence 0 or 2 nM vincristine for 48 h. The number of surviving cells was then determined using the CeIlTiter.TM. AQ.sub.ueous assay system from Promega Corporation (Madison, Wis.). The percentage of cells killed was calculated as the percentage decrease in 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium metabolism compared with control cultures. Inhibition of MRP was manifested as the ability of the compound to potentiate the cytotoxicity of vincristine toward the HL-60/ADR cells.

Detailed Description Paragraph Center (2):
Evaluation of Reversal of MDR Mediated by P-Glycoprotein or MRP.

CLAIMS:

2. A method for preventing drug resistance or enhancing the therapeutic efficacy of an antiproliferative drug in a patient undergoing chemotherapy, said method comprising administering to said patient a pharmaceutical composition according to claim 1 in an amount effective to attenuate drug resistance.

5. A method for preventing drug resistance or enhancing the therapeutic efficacy of an anti-infective agent in a patient undergoing chemotherapy, said method comprising administering to said patient a pharmaceutical composition according to claim 1 in an amount effective to attenuate drug resistance in the infecting organism.

7. A method for preventing drug resistance or enhancing the therapeutic efficacy of an antiproliferative drug in a patient undergoing chemotherapy, said method cqmprising administering to said patient a pharmaceutical composition according to claim 6 in an amount effective to attenuate drug resistance.

10. A method for preventing drug resistance or enhancing the therapeutic efficacy of an anti-infective agent in a patient undergoing chemotherapy, said method comprising administering to said patient a pharmaceutical composition according to claim 6 in an amount effective to attenuate drug resistance in the infecting organism.

12. A method for preventing drug resistance or enhancing the therapeutic efficacy of an antiproliferative drug in a patient undergoing chemotherapy, said method comprising administering to said patient a pharmaceutical composition according to claim 11 in an amount effective to attenuate drug resistance.

15. A method for preventing drug resistance or enhancing the therapeutic efficacy of an anti-infective agent in a patient undergoing chemotherapy, said method comprising administering to said patient a pharmaceutical composition according to claims 11 in an amount effective to attenuate drug resistance in the infecting organism.

17. A method for preventing drug resistance or enhancing the therapeutic efficacy of an antiproliferative drug in a patient undergoing chemotherapy, said method comprising administering to said patient a pharmaceutical composition according to claim 16 in an amount effective to attenuate drug resistance.

20. A method for preventing drug resistance or enhancing the therapeutic efficacy of an anti-infective agent in a patient undergoing chemotherapy, said method comprising administering to said patient a pharmaceutical composition according to claim 16 in an amount effective to attenuate drug resistance in the infecting organism.

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End of Result Set

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L36: Entry 9 of 9 File: USPT Mar 21, 1995

DOCUMENT-IDENTIFIER: US 5399483 A
TITLE: Expression of MDR-related gene in yeast cell

DATE FILED (1):
19921118

Priority Application Date (1):
19890330

CLAIMS:

6. A method of screening multidrug-resistant antagonists which comprises screening substances capable of inhibiting the binding of azidopine to the P-glycoprotein in the cell membranes of the composition of claim 5.

7. A method of screening multidrug-resistant antagonists which comprises screening substances capable of inhibiting the binding of azidopine to the P-glycoprotein in the cell membrane of the transformed yeast of claim 1.

8. A method of screening multidrug-resistant antagonists which screening substances capable of inhibiting the binding of azidopine to the P-glycoprotein in the cell membrane of the transformed yeast of claim 4.

L7 ANSWER 87 OF 141 MEDLINE DUPLICATE 52
ACCESSION NUMBER: 96191303 MEDLINE
DOCUMENT NUMBER: 96191303 PubMed ID: 8619891
TITLE: Reduction of expression of the **multidrug resistance** protein (**MRP**) in human tumor cells by antisense phosphorothioate oligonucleotides.
AUTHOR: Stewart A J; Canitrot Y; Baracchini E; Dean N M; Deeley R G; Cole S P
CORPORATE SOURCE: Cancer Research Laboratories, Queen's University, Kingston, Ontario, Canada.
SOURCE: BIOCHEMICAL PHARMACOLOGY, **(1996 Feb 23)** 51 (4) 461-9.
Journal code: 9Z4; 0101032. ISSN: 0006-2952.
PUB. COUNTRY: ENGLAND: United Kingdom
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LANGUAGE: English
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ENTRY MONTH: 199606
ENTRY DATE: Entered STN: 19960620
Last Updated on STN: 19970203
Entered Medline: 19960613

AB Multidrug resistance protein (MRP) is a member of the ATP-binding cassette superfamily of transport proteins which has been demonstrated to cause multidrug resistance when transfected into previously sensitive cells. Sixteen eicosomeric oligonucleotides complementary to different regions along the entire length of the MRP mRNA reduced MRP mRNA and protein levels in drug-resistant small cell lung cancer cells that highly overexpress this protein. In MRP-transfected HeLa cells that express intermediate levels of MRP, one oligonucleotide, ISIS 7597, targeted to the coding region of the MRP mRNA, **decreased** the levels of MRP mRNA to < 10% of control levels in a concentration-dependent manner. This effect was rapid but transient with a return to control levels of MRP mRNA 72 hr after treatment. A double treatment with ISIS 7597 produced a sustained inhibition, resulting in a greater than 90% reduction in MRP mRNA for 72 hr and a comparable **decrease** in protein levels. Increased sensitivity to doxorubicin was observed under these conditions. Northern blotting analyses using two DNA probes corresponding to sequences 5' and 3' of the ISIS 7597 target sequence, respectively, revealed the presence of low levels of two smaller sized RNA fragments as expected from an RNase H-mediated mechanism of action of the antisense oligonucleotide. These studies indicate that a specific reduction in MRP expression can be achieved with antisense oligonucleotides, a finding that has potential implications for the treatment of drug-resistant tumors.

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L22: Entry 1 of 18 File: USPT Aug 21, 2001

DOCUMENT-IDENTIFIER: US 6277981 B1
TITLE: Method for design and selection of efficacious antisense oligonucleotides

DATE FILED (1):
19980702

Brief Summary Paragraph Right (10):
Thierry et al. (1993, Biochem. Biophys. Res. Commun. 190:952-960) compared the efficacy of ASOs which were complementary to either the 5'-end of the coding region of or to a single-stranded loop in the mRNA encoded by the multidrug resistance gene mdr1. The results obtained by these investigators indicate that the oligonucleotides targeted to the single-stranded loop were more efficacious and specific than the oligonucleotides targeted to the 5'-end coding region. However, Laptev et al. (1994, Biochem. 33:11033-11039) obtained results which were not consistent with that suggestion. Laptev et al. concluded that the most efficacious ASOs were those which were complementary to mRNA sequences that were predicted to form clustered double-stranded secondary structures.

Detailed Description Paragraph Table (3):
TABLE 3 Identifier Inhibitory Oligonucleotide # (Ref. #) mRNA Sequence (listed 5'-3') tested A. ASOs comprising a TCCC motif, followed by C OL(1)p53.sup.1 Human p53(ORF) CCTGCTCCCCCCTGGCTCC hum. trials ISIS 1939.sup.2,3 Human ICAM-1(3'-UTR) CCCCCACCACTTCCCCTCTC 45 GM 1508.sup.4 Human ICAM-1(3'-UTR) CCCCCACCACTTCCCCTCTCA 39 ISIS 4189.sup.5 Murine PKC-.alpha.(AUG) CAGCCATGGTTCCCCCCAAC 20 ISIS 4730.sup.2 Human E-selectin(3'-UTR) TTCCCCAGATGCACCTGTTT 18 ISIS 11300.sup.6 Rat PKC-.alpha.(ORF) GACATCCCTTTCCCCCTCGG 13 C15.sup.7 1.19CAT(5'-UTR) GATCCCCGGGTACCGA 13 ISIS 3890.sup.8 Human PKC-.alpha.(AUG) GTCAGCCATGGTCCCCCCCC 20 Oligo 7.sup.9 Xenopus Xklp-1 ATGCCCTCATCCTTCCCCCCAT >9 B. ASOs comprising a TCCC motif, followed by A G 3139.sup.10 Human bcl-2 (ORF GTTCTCCCAGCGTGTGCCAT hum. trials GM 1534.sup.4 Human VCAM-1(5'-UTR) AACCCTTATTTGTGTCCCACC 28 ODN 2309.sup.11 Murine tPA (5'-UTR) GTCCCAAGAGTTGAGGAG 18 ISIS 3466.sup.12 Human p120 (3'-UTR) CACCCGCCTTGGCCTCCCAC 18 C. ASOs comprising a TCCC motif, followed by G ISIS 5132.sup.13 Human C-raf TCCCGCCTGTGACATGCATT hum. trials ISIS 5995.sup.14 Human MDR-1 (AUG) CCATCCCGACCTCGCGCT 32 T 195.sup.15 Human TNF (ORF) CCACGTCCCGGATCATGC 15 D. ASOs comprising a TCCC motif, followed by T 4484-4503.sup.16 Human HIV (SA) TCTGCTGTCCCTGTAATAAA 20 ISIS 3801.sup.3 Human VCAM AACCCAGTGCTCCCTTTGCT 15 E. ASO comprising a TCCC motif at the 3'-end thereof ISIS 3522.sup.17 HumanPKC-.alpha.(AUG) AAAACGTCAGCCATGGTCCC 20

CLAIMS:

1. A method of making an antisense oligonucleotide for inhibiting expression of a gene in an animal, the method comprising

identifying an RNA molecule corresponding to the gene, wherein the RNA molecule comprises a GGGA motif; and

synthesizing an oligonucleotide complementary to at least a portion of the RNA molecule, the portion comprising the motif, wherein the portion is selected on the basis of the presence of the motif in the portion, whereby the oligonucleotide is efficacious for inhibiting the gene.

12. The method of claim 1, wherein the RNA molecule is an mRNA of the gene.

13. A method of making an antisense oligonucleotide for inhibiting expression of a gene in an animal, the method comprising

designing a nucleotide sequence that is complementary to a portion of an RNA molecule corresponding to the gene, the portion including a GGGA motif, wherein the portion is selected on the basis of the presence of the motif in the portion, and

synthesizing an oligonucleotide having the nucleotide sequence, whereby the oligonucleotide is an antisense oligonucleotide efficacious for inhibiting expression of the gene.

(FILE 'HOME' ENTERED AT 20:21:08 ON 02 MAY 2002)

FILE 'MEDLINE, BIOSIS, CANCERLIT, LIFESCI, BIOTECHDS' ENTERED AT 20:21:41
ON 02 MAY 2002

L1 1483 S (SEMAPHORIN(W)D) OR (B(W)94) OR (MEL(W)14) OR (24(W)P(W)3) OR
L2 6 S L1 AND ((DRUG OR MULTIDRUG)(W)RESISTAN?)
L3 5 S L2 AND PY<2000
L4 4 DUP REM L3 (1 DUPLICATE REMOVED)
L5 3 S (1997 AND 94 AND 14713)/SO
L6 1 DUP REM L5 (2 DUPLICATES REMOVED)
L7 68 S SEMAPHORIN D
L8 26 S L7/TI
L9 23 S L8 AND PY<2000
L10 15 DUP REM L9 (8 DUPLICATES REMOVED)
L11 512 S COLLAPSIN
L12 2 S L11 AND ((DRUG OR MULTIDRUG)(W)RESISTAN?)
L13 3 S B(W)94/TI
L14 123 S B(W)94
L15 51 S B94
L16 33 S L15 AND PY<2000
L17 15 DUP REM L16 (18 DUPLICATES REMOVED)
L18 136 S (MEL-14)(4A)(ANTIGEN#)
L19 132 S L18 AND PY<2000
L20 22 S L18/TI
L21 10 DUP REM L20 (12 DUPLICATES REMOVED)
L22 73 S 24P3
L23 44 S L22/TI
L24 18 DUP REM L23 (26 DUPLICATES REMOVED)
L25 265 S PROLIFERIN#
L26 112 S L25/TI
L27 98 S L26 AND PY<2000
L28 39 DUP REM L27 (59 DUPLICATES REMOVED)

FILE 'MEDLINE, BIOSIS, CANCERLIT, LIFESCI, BIOTECHDS' ENTERED AT 16:11:00
ON 03 MAY 2002

L1 5946 S PLF OR MRP OR (MITOGEN(W)RE?(W)(PROTEIN# OR PEPTIDE# OR POLYP
L2 2984 S L1(S)(DRUG# OR MULTIDRUG)(A)RESISTAN?
L3 1960 S L2 AND ((DRUG# OR MULTIDRUG)(A) RESISTAN?)/TI
L4 802 S L2/TI
L5 761 S L4 AND PY<2001
L6 310 S L5 AND (MODULAT? OR DECREAS? OR ANTAGONI? OR REVERS?)
L7 141 DUP REM L6 (169 DUPLICATES REMOVED)